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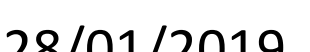
Guide to the quality and safety of tissues and cells for human application

4th edition

Draft



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Chapter 1: Introduction

We are entering a new age of medical and biotechnological progress. Medical procedures that were unimaginable a generation ago are a reality today. One aspect of the recent and rapid advances in biological and medical research is that human tissues and cells are being used increasingly in new ways. Many of these developments, such as advances in transplantation therapy or in medically assisted reproduction (MAR), have unquestionable benefits. However, using human tissues and cells in different ways also raises questions of safety, quality and efficacy, and presents new ethical dilemmas.

Tissue from one deceased donor may be transplanted into as many as 100 individuals. Some other tissues and cells can be provided only by living donors, as long as this procedure does not risk serious harm to the donor or endanger the donor's life. Transplantation of tissues and cells can range from life-saving treatments (e.g. in the treatment of catastrophic burns) to quality-of-life improvements. In addition, donated gametes and embryos may help fulfil a person's wish to have children.

Some tissues are used practically unaltered from the condition in which they were removed from the donor. Deceased donor corneas, for example, are used to restore sight, heart valves replace damaged ones and extend life, tendons and ligaments may be used for the treatment of sporting injuries or to repair degenerative defects, and skin can be employed to cover major burns or support the healing of ulcers. Other tissues, however, are processed into products that are almost unrecognisable as bodily material. Skin, for example, may be cut into conveniently sized dressings, incorporated into sprays or gels, or decellularised for use in various surgical procedures. Bone can be processed into hundreds of different products and distributed via a global medical market for use in orthopaedics (general and oncology), sports medicine, craniofacial/maxillofacial/dental surgery and neurosurgery. Cellular components of bone may be removed entirely and even the calcium may be removed to promote incorporation and tissue regeneration. Bone allografts may be precision-cut and sized, and bone can also be supplied in soft, pliable or injectable forms. If a deceased donor has consented to the use of any part of their body for the treatment of others (or their relatives have authorised this to fulfil the donor's wishes), then many tissues – including bone, heart valves, skin, corneas, ligaments, cartilage, connective and adipose tissue, glands and nerves – can be used for therapeutic purposes.

In contrast, amniotic membranes and placenta, parathyroid tissue and skull bone are donated by living persons. Additionally, femoral heads removed during an operation to replace a hip joint and heart valves from patients receiving a heart transplant are sometimes processed and 'recycled'. In addition, many types of cell can be donated (some during life and some after death) and submitted to different degrees of manipulation before application in humans. Examples include haematopoietic progenitor cells (e.g. bone marrow, peripheral blood progenitor cells, umbilical cord blood), somatic cells (e.g. peripheral blood cells, keratinocytes, chondrocytes, hepatocytes), mesenchymal stromal cells and limbal stem cells. Oocytes, sperm, ovarian or testicular tissue and embryos can be used in MAR procedures to achieve pregnancy.

Human tissues and cells could also be the potential starting material for much more complex products in the future.

1.1. Scope and purpose of this Guide

This is the 4th edition of the Council of Europe *Guide to the Quality and Safety of Tissues and Cells for Human Application*. This Guide has two main objectives:

a. It aims to provide sound information and guidance – for all professionals involved in donation, banking, transplantation and other clinical applications of tissues and cells – to optimise the quality and minimise the risks of these complex procedures. All material of human origin carries risks of disease transmission that must be controlled by application of scrupulous criteria of donor

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selection and testing, and comprehensive systems to assess quality. The idea behind this Guide is to help professionals on a practical level by providing generic guidance that will help improve the rate of successful clinical application of tissues and cells.

b. This Guide includes ethical principles and guidelines to be considered for the donation and human application of tissues and cells.

The field of tissue and cell donation and banking is now highly regulated in many countries. In the European Union (EU), several directives describe the requirements and have been transposed into the national legislation of the 28 EU member states. This Guide refers to those requirements where appropriate, but goes beyond them to describe generally accepted good practice at a technical level and includes some consideration of ethical issues. Therefore, it will be useful as a source of practical information for those working within the EU legislative framework and those working within national legal frameworks in all Council of Europe member states and beyond. The Guide does not provide any guidance on how human tissues and cells are or should be regulated, so users of this Guide are advised to carefully consider the national legal requirements that apply to the activities they are undertaking.

According to the World Health Organization (WHO) *aide-mémoire* on the donation and transplantation of tissues and cells [1], national Health Authorities are responsible for ensuring that the donation, banking and human application of tissues and cells are promoted, regulated and monitored appropriately in the interests of patient safety and public transparency. More specifically, they are responsible for ensuring that:

a. an appropriate legislative/regulatory framework is in place;
b. national/international practice standards have been defined;
c. there is inspection/authorisation of screening, testing, procurement, processing, storage, distribution, import and export;
d. there are programmes for vigilance and surveillance of adverse outcomes;
e. there is monitoring and reporting of donation, processing, storage, distribution and import/export activity.

In this Guide, the term 'Health Authority' is used throughout to refer to a body that has been delegated the responsibility for these activities on a national or regional basis by their government. Other similar terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it. It should be noted that in some countries, the activities described in this Guide may be controlled by different Health Authorities – e.g., separate authorities may regulate MAR and the donation, banking and human application of other tissues and cells. Unless otherwise indicated, the term 'member states' applies to member states of the Council of Europe.

Human tissues and cells also play a key part in medical research. In clinical trials of new medicines, for example, vital information about the effects of the medicine on an individual can be obtained from samples of tissues or cells and other materials provided by research participants. However, tissue is also used much more widely in medical research, from early drug 'discovery' (such as using human tumour samples to discover possible targets for treatment) to later clinical development whereby samples may be used to identify which subgroups of patient populations respond best to a new medicine. Additionally, current research aims to develop artificial tissue that could alleviate the shortage of tissue available for human application. These forms of 'basic' research using human tissue still have an ultimately therapeutic goal in mind. However, important though all these possibilities are, this Guide covers only tissues and cells used for current therapeutic purposes.

Similarly, all tissues and cells procured and applied within the same medical procedure are outside the scope of this Guide.

Finally, a glossary of terms is provided in **Appendix 3**.

This book is the result of the collective effort and expertise gathered by experts nominated by the member states and professional associations in the field (see **Appendix 34**), as well as by the members and observers of the European Committee of Experts on Organ Transplantation (CD-P-TO), for which see **Appendix 35**.

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208 For matters dealing with the use of organs and blood or blood products, see the *Guide to the*
209 *quality and safety of organs for transplantation* and the *Guide to the preparation, use and quality*
210 *assurance of blood components* [2], both published by the Council of Europe.

211 1.2. Brief history of the application and banking of tissues and cells 212 of human origin

213 The best documented accounts of early transplants deal with skin transplantation, though the success or
214 failure of these procedures has not been well documented. The first reliable account is that of the Indian
215 surgeon Sushruta in the 2nd century BC, who used autografted skin transplantation for a nose
216 reconstruction (rhinoplasty). Centuries later, the Italian surgeon Gasparo Tagliacozzi carried out
217 successful skin autografts, but he consistently failed with allografts, offering the first suggestion of
218 rejection several centuries before that mechanism could be understood. He attributed it to the "force and
219 power of individuality" in his 1596 work *De Curtorum Chirurgia per Insitionem*. Orthopaedic surgeons
220 refer to the origin of their discipline as 1668 when Job van Meekeren reported on the grafting of bone
221 from a dog's skull to correct a defect in a soldier's cranium. It was not until 1869 that the first completely
222 documented fresh human-skin allograft was carried out by the Swiss surgeon Jacques Reverdin.
223 The first successful full-thickness corneal transplant, a keratoplastic operation, was carried out in
224 1905 by Eduard Zirm at Olomouc Eye Clinic in Moravia (now Czech Republic). Pioneering work in the
225 surgical technique of transplantation was done in the early 1900s by the French surgeon Alexis Carrel,
226 together with Charles Guthrie, who developed techniques for suturing arteries and veins. Their skilful
227 anastomosis operations and new suturing techniques laid the groundwork for later transplant surgery,
228 and Alexis Carrel won the 1912 Nobel Prize in Physiology or Medicine for his work in the field. Major
229 steps in skin transplant occurred during the First World War, notably through the work of Harold Gillies
230 in Aldershot, UK. Among his advances was the tubed pedicle graft, which maintained a fleshy
231 connection from the donor site until the graft established its own blood supply.
232 Bone is the oldest tissue transplant on record and the most common tissue transplanted today. The
233 first bone transplant recorded in modern times occurred in Scotland in 1878 when Sir William Macewen
234 removed an infected humerus from a 12-year-old boy and replaced it with three allografts from an
235 amputated tibia from another child with rickets. In 1907, Erich Lexer in Berlin developed a procedure to
236 remove a whole knee joint from an amputee in one operating room and transport the 'warm' graft to an
237 adjacent operating room for immediate transplant into the recipient. Five years later, Alexis Carrel's
238 work predicted the storage of tissues for future transplantation, and surgeons began to use bones and
239 developed their own 'bone banks'. These pioneers included Inclan in Cuba, Bush, Wilson and Hibbs in
240 the USA, Hult working in Sweden, Judet in France, and Klen in what was then Czechoslovakia. Most
241 of these early bone banks were simply refrigerators and, later, freezers, but greater sophistication was
242 developed by bone banks in Leeds (UK), Berlin, Athens and Warsaw. When long-term freezer storage
243 of long bones became feasible, limb-sparing surgery using this type of bone allograft to avoid
244 amputations in the treatment of malignant skeletal tumours became popular. Burrwell (UK), Parrish and
245 Mankin (USA) and Ottolenghi (Argentina) published their results.
246 The orthopaedic profession realised that, if very large segments of bone could be transplanted
247 successfully, smaller segments could also be used. This resulted in a very large increase in the use of
248 bone allografts. Tissue-storage methods were developed further during the 1950s by Hyatt at the US
249 Navy Tissue Bank in Bethesda, Maryland, where they adapted methods of lyophilisation from the food
250 preservation industry and applied the process to the preservation of bone and skin, which could then be
251 easily stored, transported and reconstituted for use when needed. This method of preservation allowed
252 bone to be stored and transported easily without any electrical or mechanical requirements, and has had
253 a profound effect on the availability and use of bone allografts. By the end of the 1990s, use of
254 musculoskeletal tissue allografts (i.e. bone, cartilage, soft tissue) had become commonplace in many
255 clinical areas. Similarly, the first deceased-donor eye bank was established in Odessa using eyes (packed
256 in glass containers) sent by rail from a trauma centre in Moscow.

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The first recorded cardiac valve transplantation was carried out in Toronto by Gordon Murray, who implanted an aortic allograft in the descending thoracic aorta to relieve aortic insufficiency in 1956. The first orthotopic transplantation of the aortic valve was undertaken by Donald Ross in London in 1962 and independently by Brian Barratt-Boyes in Auckland, New Zealand, a few weeks later. Pulmonary and mitral valves were first used as allografts in subsequent years, with the pulmonary autograft procedure being carried out first in 1967.

After the atomic bomb explosion in Japan that ended the Second World War, many scientists began to explore ways of protecting humans from radiation. The first experiments were done in mice and later in dogs by E.D. Thomas. As early as 1956, the idea that bone-marrow transplants might exert a therapeutic effect against malignancies was proposed by Barnes and Loutit, who observed an anti-leukaemic effect of transplanted spleen cells in experimental murine models. In 1959, the first human bone-marrow transplants gave proof of concept that infusions of bone marrow could provide haematological reconstitution in lethally irradiated patients with acute leukaemia. E.D. Thomas performed transplants in two patients with advanced acute lymphoblastic leukaemia, with a syngeneic graft after high-dose total body irradiation; the grafts were successful but the patients died a few months later of relapse. G. Mathé administered allogeneic bone marrow for the treatment of several patients who had suffered accidental exposure to irradiation, and most survived with autologous reconstitution. In 1965, Mathé was the first to describe long-term engraftment of sibling bone marrow, thereby demonstrating chimerism, tolerance and an anti-leukaemic effect. Although the transplant itself was successful, the patient eventually died of varicella with chronic graft *versus* host disease (GvHD). In 1970, M. Bortin reported 203 transplants carried out between 1958 and 1968, with only three patients alive at the time of the report. The major causes of death were graft failure, GvHD and relapse. After these disappointing results, few centres persisted and the number of transplants declined sharply.

Major progress came from the discovery of the human leukocyte antigen (HLA) system by J. Dausset and J.J. Van Rood. Selection of HLA-identical siblings as bone-marrow donors diminished the risk of rejection and GvHD. Using animal models, R. Storb and E.D. Thomas developed the model of total body irradiation for conditioning (in dogs) and the use of methotrexate for GvHD prevention. In mice, G. Santos showed that the use of cyclophosphamide could add immuno-suppression to the myeloablation of total body irradiation. He was also the first to use busulfan instead of total body irradiation. In 1988, the first successful cord blood stem-cell transplant was done to treat a child with Fanconi's anaemia with cells from his healthy HLA-identical sibling (related) donor. The first unrelated bone-marrow registry was established in London in 1973 by Shirley Nolan, whose son was diagnosed with Wiskott–Aldrich syndrome. After this first donor recruitment drive, the number of bone-marrow and peripheral haematopoietic progenitor cell donors has increased all over the world, with more than 25 million donors now registered, including more than 600 000 cord blood donors [3].

Transplantation of pancreatic islets has been carried out in humans since 1990 [4]. However, it was not until 1999 that the first successful transplant of pancreatic islets, using the so-called Edmonton Protocol, was undertaken by James Shapiro [5]. European centres became active around the same period, but their transplant recipients had complications of type-I diabetes that could not be managed with insulin injections. The advantage of the Edmonton Protocol was that it allowed restoration of the finely tuned regulation of glucose metabolism through appropriate insulin production by transplanted islets. In 2005, the first pancreatic islet transplant from a living donor – from a 56-year-old woman to her 27-year-old diabetic daughter – resulted in transplanted cells producing insulin within minutes after transplantation.

On 25 July 1978, Louise Brown, the first *in vitro* fertilisation (IVF) baby, was born in Oldham, UK [6]. Her birth was the result of the collaborative work of Patrick Steptoe and Robert Edwards. Since then, this research area has seen major improvements in the laboratory – e.g. cryopreservation of gametes and embryos, intracytoplasmic sperm injection (ICSI) [7], pre-implantation genetic diagnosis [8] and clinical management (such as improvements to methods for ovarian stimulation and embryo culture conditions) – thereby leading to a considerable increase in the use of assisted reproductive technologies (ART). To date, more than 5 million babies have been born worldwide through MAR. Data

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from the International Committee Monitoring Assisted Reproductive Technologies (ICMART) show that around 1.5 million ART cycles are now performed globally each year, with around 350 000 babies born as a result [9]. This number continues to rise.

1.3. Benefits and risks of human application of tissues and cells

Progress in the medical sciences has made it possible to effectively transplant human cells and tissues from one person into another. Transplantation of tissues, such as corneas, cardiovascular tissues, bone, tendons and skin, are all well-established therapeutic techniques. Cornea and musculoskeletal tissues are the most commonly transplanted, outnumbering organ transplants by more than tenfold. Although not all of these tissues are necessarily life-saving, such transplants nevertheless offer major therapeutic benefits to a wide range of patients. Indeed, demand is rapidly increasing for bone transplantation, particularly for secondary revision of hip-replacement operations, as well as for skin treatment of severely burned patients. Successful transplantation, even when not acutely life-saving, offers recipients major improvements in their quality of life.

The main differences between organ and tissue transplants are summarised in Table 1.1. Because of these differences, donor-selection criteria for tissue donors can often be more stringent.

Table 1.1. The main differences between organ and tissue transplants

Organs	Tissues
Usually life-saving	Usually not life-saving but life-enhancing
Donor pool is small	Donor pool is larger
Time to implantation is usually measured in hours and the organs cannot be preserved for future use	Time to implantation can be measured in days or years, depending on the tissue and the preservation method applied
Donor can supply only a small number of recipients	One donor's tissues can be transplanted into many patients, so donor-selection failures can affect many recipients
Cannot be sterilised or exposed to robust decontamination processes	Tissues and cells can often be subject to decontamination and/or sterilisation methodologies
Often the only therapeutic option	Alternative treatments usually available

Cells fall somewhere between organs and tissues in this comparison. They are intended to be life-saving and are usually transplanted on the basis of one donor to one recipient. However, they can be processed to some extent, though not sterilised, and they can be stored for extended periods. Where bone marrow is donated by an unrelated donor for a specific recipient and transplanted without freezing, the situation is very analogous to organ transplantation. In contrast, when cord blood is donated to a public bank, stored for years and possibly selected later for transplant to a matching recipient, the situation is more analogous to tissue banking.

In practice, the decision to transplant any donor-derived tissues or cells will always be based on a clinical assessment of the risk *versus* the benefit to the patient, taking any alternative potential therapies into consideration. This is because any human application of tissues and cells carries not only process-related risks, but donor-related disease-transmission risks. The factors influencing the clinical outcome are complex because there is an interaction between two different biological systems, namely, those of the donor and the recipient. Therefore, when assessing the risk of human application of tissues and cells, both donor and recipient should be considered. In both cases, the potential benefits of the transplant procedure should outweigh the risks. Transparent communication and good collaboration between Health Authorities, tissue establishments and clinicians treating patients are vitally important in any donation process.

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Some of the most widely used tissues and cells, and their benefits for transplant recipients, are listed in Table 1.2.

Table 1.2 Most widely used tissues and cells: the benefits for the transplant recipient

Tissues and cells	Function	Benefits for the recipient
Amniotic membrane	Forms the amniotic sac, filled with amniotic fluid, which surrounds and protects the foetus; transfers oxygen and nutrients from mother to foetus	Used in burns and wound healing (to reduce surface inflammation, scarring and pain in surgical applications), in certain types of ulcers and in oral, maxillofacial and ocular surface surgery
Bones and cartilage	Support the body and protect vital organs	Used to repair or stabilise the spine and other bones and cartilage damaged by degeneration, trauma, cancer or birth defects; also used in oral surgery and to fill bone cavities or other areas where bone mass has been lost
Corneas/eyes	Cornea and sclera together form the outer coat of the eye: the cornea is transparent and lets light into the eye; the white sclera is opaque	Indicated for visual problems caused by damage or deterioration of the front part of the ocular globe; if whole eyes are donated, the corneas can be used in transplants for corneal blindness and the sclera can be used for reconstructive and glaucoma surgery
Gametes, reproductive tissues and embryos	Generate a new human being	Used primarily for the treatment of infertility and to achieve pregnancy and live birth in single women and same-sex couples; can be stored to preserve fertility or even re-establish gonadal function (in the case of reproductive tissues); assisted reproductive technologies can also be applied to avoid transmission of some genetic or infectious diseases
Fascia	Fibrous tissue that covers muscles	Used to repair tendons, muscle, ligaments and deformities
Haematopoietic progenitor cells (bone marrow, peripheral blood progenitor cells and cord blood)	Haematopoiesis	Used for the treatment of haemato-oncologic disorders, and genetic and autoimmune diseases
Heart valves	Direct the flow of blood in the heart	Used for patients with valve defects, especially in children
Pancreatic islets	Contain beta cells, which are responsible for insulin production	A transplantation method that restores an adequate mass of insulin-producing beta cells in patients with diabetes
Pericardium	Forms protective lining around the heart	Used for replacement of *dura mater* in the brain and for eye surgery
Skin	Protects the body against injury, infection and dehydration	Used for the treatment of burns patients, certain types of ulcer, abdominal wall repairs and reconstructive or plastic surgery
Tendons	Attach muscle to bone	For use in joint injuries
Veins and arteries	Provide a structure for the flow of blood through the body	Replace blood vessels that are damaged by disease, trauma or prolonged dialysis treatment. Also used in bypass surgery to re-route blood flow

With regard to the risks associated with the human application of tissues and cells, Article 6 of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin clearly establishes that:

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> all professionals involved in organ or tissue transplantation must take all reasonable measures to minimise the risks of transmission of any disease to the recipient and to avoid any action which might affect the suitability of an organ or tissue for implantation.

Careful evaluation – of the donor's medical case history, travel history, behavioural risks and history of malignancies – is necessary to keep the risk of transmission of infections or malignancies to the recipient as low as possible. These risks are covered in Chapter 4. Specific criteria regarding tissues and cells are discussed in detail in the relevant chapters of Part B of this Guide.

Only tissues and cells recovered, processed, stored and distributed within well-controlled quality management systems of donation, processing, storage and distribution are likely to function satisfactorily and to reach an acceptable level of safety. The donor-selection criteria and the conditions of processing and preservation are crucial parameters that need to be tightly controlled. Therefore, any organisation involved in these processes should implement a comprehensive quality-management system. Management commitment and support are essential for the development, implementation and monitoring of a quality system to ensure continuous improvement. All staff should understand the importance of quality and their role in achieving it consistently.

In summary, human application of tissues or cells can confer great benefit for a patient, but it is not without risk. In exceptional cases, a donation of tissues or cells that does not meet all the necessary safety or quality requirements may be used for human application for a particular patient. This may occur, for instance, where the transplant is likely to be life-saving and the alternative options for treatment of that patient carry a poor prognosis. Similarly, couples undergoing MAR treatments often use gametes that would not meet selection criteria for non-partner procedures (e.g. gametes from an infected partner, low-quality sperm, gametes with a well-known risk of transmission of a generic disease). Ultimately, patients contemplating use of any donated tissues or cells should discuss the risks and benefits of surgery/therapy with their surgeon/physician and make the decision that is best for them.

1.4. The process of donation of tissues and cells and their application in humans

Donation of tissues and cells and their application in humans continue to be fast-moving fields. Such rapid developments bring their own challenges. These challenges include: control of all crucial technical activities and services (procurement, transportation, processing, preservation, quality control, storage) that enable tissues and cells to be removed from one person and transferred to another body, reimbursement of expenses and service charges, safeguards from exploitation or misuse (e.g. formal requirements for consent from the potential donor before procurement of tissues or cells) and the complex chain of intermediaries (people and institutions) in the process of donation and human application.

The process of donation of tissues or cells from a deceased donor is, in many respects, quite different from the process in living donors; but, in all cases, a complex network of interactions underlies the many ways in which human material may be provided by one person for the benefit of others. Some of these complex links, using the example of a deceased donor, are summarised in Figure 1.1.

We can conceptualise the entire process in terms of organisation and workflows. In the case of donation after death, transplantation can take place only if trained professionals are available to talk to the family of the deceased potential donor, if there is the necessary infrastructure to procure tissues within a given timeframe and process them, if transport services exist to transport tissues appropriately and if surgeons are available to carry out tissue transplantation into the recipient. Similarly, living donation is possible only if professionals recruit and evaluate potential donors, and adequately trained personnel carry out the processes that will generate the medical products used to treat patients.

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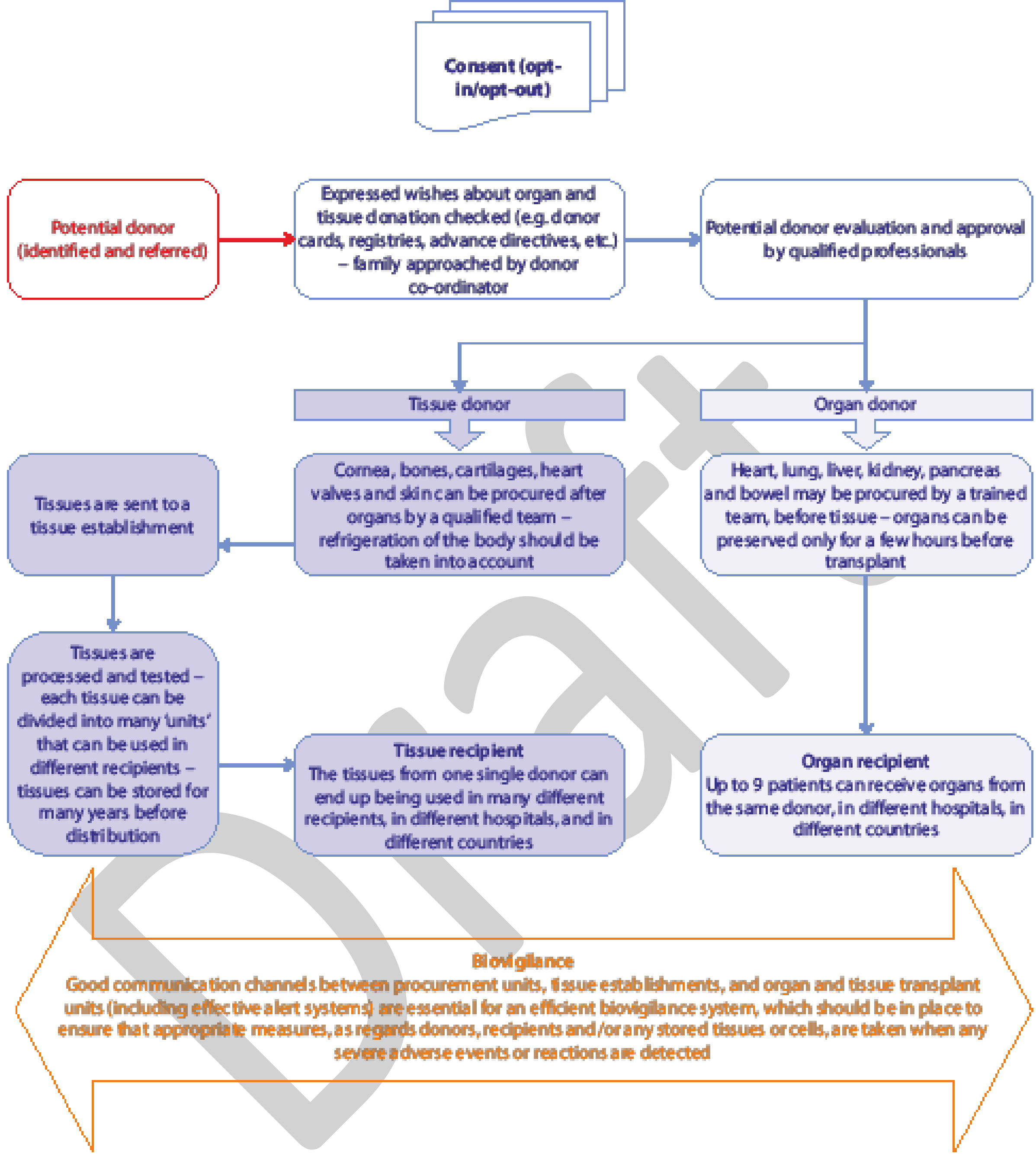
Figure 1.1. Complex links between donors and recipients in the context of donation after death



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Tissue establishments play a central role in modern medicine by providing material for treatment and research. Tissue banking and cell banking are increasingly interconnected as part of the complicated networks that now connect the sources and recipients of donated bodily material, and the many intermediaries involved in processing the material to facilitate its use by clinicians.

Centralised management of tissue and cell donations could be the ideal scenario. However, tissues and cells can be provided from public organisations and private companies. Co-operation between establishments that store tissues and cells may be relatively limited. National and international efforts have focused on good practice for tissue establishments without usually providing a mechanism for comprehensive, nationwide sharing of donated material. In the meantime, an industry based on the supply of human tissue and cells has evolved worldwide, with multiple providers competing in a market driven by, among other things, biotechnology companies, pharmaceutical companies and private clinics.

412 Thus, the flows involved between the original source or donor of the material, the amount of processing
413 of the material involved and the commercial nature of some of those transactions are becoming ever
414 more complex.
415 It is important to emphasise how consideration of policy surrounding donation must now take into
416 account these complex flows and multiple intermediaries [10]. Awareness is needed of the central part
417 that must be played by organisations and organisational structures in the donation and subsequent use
418 of bodily material. Everyone involved needs to understand how the process includes, for example, the
419 creation of professional roles such as 'donor co-ordinators', the extent to which they are expected to
420 maximise opportunities for donation, how these professionals approach potential donors and form
421 relationships with them, how well one part of the system links with another and where responsibility is
422 seen to rest, and the way professionals in different fields interact and co-operate with one another.
423 Awareness of this also points to added complexities in the form of legal agreements, liabilities and
424 obligations that may arise where donated material is transformed, banked or otherwise handled as a
425 commodity by successive intermediaries.
426 The increasing possibilities in using many forms of bodily material to benefit others in medical
427 treatment has brought about increased pressure in member states to meet demand. There is a continual
428 need to recruit new tissue and cell donors to maintain an adequate supply. Shortages of supply may
429 affect particular subgroups of the population more than others because of the need to match material
430 according to immunological criteria or age. 'Demand' for material is inherently variable; as scientific
431 developments make more treatments possible, the demand for that treatment is likely to increase,
432 whereas the development of alternatives may lead to reduced demand. Public expectations of what
433 medical science can achieve may serve to put further pressure on demand.
434 Talking in terms of 'supply' and 'demand' may resonate with the experience of many
435 professionals and patients (potential recipients), who are only too aware of the impact of any shortage
436 in supply. This feature is exacerbated in situations in which the requirement for a high degree of
437 matching or phenotypical similarity between donor and recipient calls for recruitment from ethnic
438 minorities and international collaboration. However, at the same time, it may imply a lack of
439 consideration of the human nature of the source of the material. It is important to emphasise when using
440 these impersonal terms that we are talking about people and people's lives.

441 1.5. Tissue banks, tissue establishments and biobanks

442 A 'tissue bank' is a term commonly used to describe an establishment that collects and stores human
443 tissues or cells for either medical research or human application.
444 Increased use of tissues and cells for human application and for research calls for terminology
445 that will distinguish between establishments that collect and store tissues and cells for one of these
446 purposes or the other. In Europe, the terms currently in use are 'tissue establishment' (for clinical
447 applications) and 'biobank' (for research applications).
448 The term 'tissue establishment' became widely used in Europe following publication of the EU
449 Tissues and Cells Directive 2004/23/EC, which defined it as:

450 a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or
451 distribution of human tissues and cells for human application are undertaken. It may also be responsible for
452 procurement or testing of tissues and cells.

453 In the field of MAR, the term 'tissue establishment' refers to the laboratories in MAR centres or clinics
454 as well as banks of gametes. These centres or clinics often also include clinical units in which the patients
455 are treated. In the context of this Guide, the term 'tissue establishment' will be used and refer to all these
456 banks, units, centres and clinics. The directive does not cover research using human tissues and cells, so
457 tissue establishments are concerned only with tissues and cells intended for human application.
458 Similarly, the directive does not cover the clinical application and practices undertaken in the clinical
459 units of MAR centres.
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461 In the USA, the American Association of Tissue Banks (AATB) uses the term tissue bank for:

462 an entity that provides or engages in one or more services involving tissue from living or deceased individuals
463 for transplantation purposes. These services include assessing donor suitability, recovery, processing, storage,
464 labeling, and distribution of tissue. [11].

465 The term 'biobank' is widely used for repositories storing human biological samples for use in research.
466 Presently, there is not an internationally agreed definition of a biobank, but the term is generally used
467 for organised collections of human biological material (blood, tissues, cells, other body fluids, DNA,
468 RNA, etc.) and associated information stored for one or more research purposes. In its glossary, the
469 Organisation for Economic Co-operation and Development (OECD) defines a biobank as "a collection
470 of biological material and the associated data and information stored in an organised system, for a
471 population or a large subset of a population" [12]. Several other definitions, as used in EU legislation or
472 guidelines, are available on the website of the EU-funded project PRIVILEGED (Privacy in Law, Ethics
473 and Genetic Data) [13].
474 In the USA, the term 'biorepository' is preferred to 'biobank'. For example, according to the
475 glossary of the National Cancer Institute, a biorepository is:

476 a facility that collects, catalogues, and stores samples of biological material, such as urine, blood, tissue, cells,
477 DNA, RNA, and protein, from humans, animals, or plants for laboratory research. If the samples are from
478 people, medical information may also be stored along with a written consent to use the samples in laboratory
479 studies. [14]

480 The biobanking field is continually evolving, and tissue establishments may become interested in
481 collecting samples for research purposes, so the terminology should also be refined to reflect these
482 changes in the future.
483 In this Guide, it has been agreed to use the term 'tissue establishment' and its definition in
484 accordance with Directive 2004/23/EC.

485 1.6. Quality and safety

486 High-quality, safe and efficacious procedures are essential for donors and recipients alike. The long-
487 term outcomes of tissue and cell donation and human application should be assessed for the living donor,
488 as well as the recipient, to document benefit and harm.
489 The level of safety, efficacy and quality of human tissues and cells for human application as health
490 products of an exceptional nature must be maintained and continually optimised. This strategy requires
491 implementation of quality systems (see Chapter 2) that include traceability (see Chapter 15) and vigilance
492 (see Chapter 16), with adverse events and reactions reported both nationally and for imported/exported
493 human products.
494 Optimising the outcome of the human application of tissues and cells entails a rules-based process
495 that encompasses clinical interventions and *ex vivo* procedures from donor selection through to long-
496 term follow-up. Under the general supervision of Health Authorities, transplant and MAR programmes
497 should monitor donors and recipients to ensure that they receive appropriate care, including information
498 about long-term risks and benefits. Evaluation of information on long-term risks and benefits is essential
499 to the consent process and for adequately balancing the interests of donors and recipients. The benefits
500 to both must outweigh the risks associated with donation and human application. Donors should not be
501 permitted to donate in clinically hopeless situations.
502 Locally organised donation, transplantation and MAR programmes should store details of their
503 activity and follow-up data in national and/or international registries. All deviations from accepted
504 procedures that could increase the risk to recipients or living donors (as well as any untoward
505 consequences of donation or human application) should be reported to, and analysed by, the responsible
506 Health Authorities.
507 Transplantation of human material that does not involve long-term medical care of the recipient
508 may not require active, long-term follow-up, though traceability should be ensured for the anticipated

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509 lifetimes of donor and recipient. Internationally agreed means of coding to identify tissues and cells used
510 in transplantation are essential for full traceability (see Chapter 14).
511 In the specific case of MAR, traceability should include the outcome of the pregnancy as well as
512 the health of the donor, recipient and newborn. It is of the utmost importance to put the welfare of donors
513 (especially with respect to non-partner oocyte donors) in a central position in determining what
514 constitutes acceptable practice. This requirement might entail additional effort in the context of cross-
515 border reproductive care. All gamete donors should be recorded in national registers, and all centres
516 should participate in the collection of national and international data. MAR centres and Health
517 Authorities should collect data on a systematic basis to follow up the long-term health effects of MAR
518 activity, including the health of the donor, recipient and newborn. Good-quality evidence on these effects
519 is essential for appropriate concern to be given to the welfare of oocyte donors in future policies. In
520 addition, there should be a limit to the number of times a woman may donate, and a minimum interval
521 between donations should be established. Ultimately, the welfare of oocyte donors should underpin any
522 consideration about donation.

523 1.7. Ethical issues

524 Human tissues and cells can be derived only from the body of a person – hence the ethical challenges
525 associated with their use. The range of tissues and cells described in this Guide makes explicit the very
526 different circumstances under which a person can donate. The person providing the material may be
527 living or deceased, the material may be used almost immediately or stored for long periods of time, and
528 the material may be used unprocessed or heavily processed. Whatever the case, handling and disposal
529 of human tissues should be carried out in a manner that shows respect for fundamental rights and for the
530 human body.
531 Ethical standards of all aspects of tissue and cell donation and transplantation have to conform to
532 the Oviedo Convention on Human Rights and Biomedicine (1997) [15] and the Additional Protocol on
533 transplantation of organs and tissues of human origin (2002) [16]. Other important guidelines to observe
534 from an ethical viewpoint are Committee of Ministers Resolution (1978) 29 on harmonisation of
535 legislation of member states relating to removal, grafting and transplantation of human substances [17],
536 the WHO Guiding Principles on human cell, tissue and organ transplantation [18], the Declaration of
537 Istanbul on Organ Trafficking and Transplant Tourism [19] and the Barcelona Principles on the use of
538 human donated tissue for ocular transplantation, research and future technologies from the Global
539 Alliance of Eye Bank Associations (GAEBA) [20].
540 Tissues donated for transplantation after death are governed by the same ethical principles as
541 organs since they enter a common pool to be used according to need and their use cannot be directed to
542 a particular individual. Cells such as those from the bone marrow can be donated by a living person and
543 directed for transplantation to another specific person. Similarly, gametes may be donated for use within
544 the couple but may also be donated to unrelated recipients for altruistic reasons.
545 For tissue donation from deceased individuals, the 'dead-donor rule' (which states that patients
546 must be declared dead before removal of any vital organs or tissues for transplantation) must be strictly
547 respected [21].

548 1.7.1. Consent

549 The Oviedo Convention states that an intervention in the health field may be carried out only after
550 the person concerned has given free and informed consent to it. This person must make a free choice in
551 the absence of any undue influence and must be given appropriate information beforehand as to the
552 intended use and nature of the intervention as well as its consequences and risks. The person concerned
553 may freely withdraw consent at any time.
554 Together with the Declaration of Istanbul, a joint initiative of the International Society for
555 Nephrology (ISN) and The Transplantation Society (TTS), the Additional Protocol to the Convention
556 on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin
557 expands these provisions further for the specific case of donation and transplantation. These provisions

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are explained further in detail in Chapter 3. Specific cases related to consent in MAR procedures are outlined in Chapter 27.

Tissues must not be removed from the body of a deceased person unless that person has been certified dead in accordance with the national law and consent or authorisation has been obtained. The removal must not be carried out if the deceased person had objected to it.

Finally, it is crucial to emphasise the importance of consent in creating and maintaining the trust of the general public in health professionals and the healthcare system as a whole. 'Medical mistrust', or mistrust of the healthcare system, is one of the reasons why people are reluctant to donate bodily material. This may be associated with concerns about consent in that the terms of the consent may be abused (for example, by using the donated material in a manner which is not in accordance with consent) and that additional material may be taken without explicit consent. Values such as honesty and trust are central in both the professional and personal relationships when donation of bodily material takes place. Therefore, it is of vital importance that the limits of the consent are clearly established, made explicit and scrupulously respected.

The recipient – and, if appropriate, the person or official body providing authorisation for the human application – must be given suitable information beforehand on the purpose and nature of the procedure, its consequences and risks, and the alternatives to the intervention.

In summary, all donation and transplantation programmes are dependent upon the goodwill and voluntary donation of relevant material from donors to continue their activity. It is, therefore, important that public confidence is maintained by standards of good practice. By engaging donor trust and commitment when obtaining consent, healthcare professionals will reduce the risk of nefarious trading and potential physical harm from the use of transplantable tissue for human application.

1.7.2. Conflicts of interest

To avoid any potential conflict of interests, physicians determining the death of a potential donor should not be directly involved in tissue or cell procurement from the donor or subsequent transplantation procedures, and nor should they be responsible for the care of any intended recipient of such tissues or cells.

Health Authorities will set out the legal standards for determining that death has occurred and specify how the criteria and process for determining death will be formulated and applied.

It is of the utmost importance that patients undergoing ART treatment should be counselled appropriately for their conditions and given realistic estimates of the prospects of success of their treatment, based on their age and specific medical circumstances. Similarly, the welfare of potential donors (especially oocyte donors) should be central in determining what constitutes acceptable practice. Gamete scarcity or financial profit should never influence the decision to accept a donor into a programme. Also, financial incentives must not be used to encourage donations because they will render women more likely to consider repeat donations or to continue donating despite potential risks to their health. Furthermore, financial incentives may lead donors to not disclose all the information necessary for a complete and adequate donor selection, thus compromising the quality and safety of the donated oocytes.

1.7.3. Financial aspects of donation and human application of tissues and cells

Discussions around how best to increase the supply of human tissues and cells often focus on questions of donor motivation: specifically, how individuals may best be encouraged to donate different forms of bodily material. Nevertheless, it is essential to recall the Oviedo Convention which, in Article 21, clearly states that the human body and its parts must not, as such, give rise to financial gain. The Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors [22] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation. This notion is reiterated in the additional protocol to that Convention, which also clearly states in its Article 21 that the human body and its parts must not, as such, give rise to financial gain or comparable advantage. The aforementioned

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provision does not prevent payments that do not constitute a financial gain or a comparable advantage, in particular:

a. compensation of living donors for loss of earnings and any other justifiable expenses caused by the removal or by the related medical examinations;
b. payment of a justifiable fee for legitimate medical or related technical services rendered in connection with transplantation;
c. compensation in cases of undue damage resulting from the removal of tissues or cells from living persons.

In the donation of any tissue or cell, removal of barriers to donation must not render a decision to donate non-altruistic. Initiatives that reduce the barriers to donation should only facilitate individuals to carry out an action that they were already inclined to take by concern for the welfare of the recipient. In this sense, the Nuffield Council on Bioethics suggests distinguishing between two types of intervention, both of which aim to increase donation by changing its costs and benefits [23]. The first type is 'altruist-focused interventions', which typically involve removal of various disincentives to act and, in doing so, remove countervailing concerns that may hinder potential donors from acting on their altruistic motivations. For the purpose of this Guide, we will call these interventions 'compensation'. The second type is 'non-altruist-focused interventions', which are targeted at potential donors who have no strong motivation to help others through donation of their bodily material and who, therefore, if they are to donate, need to be provided with different reasons for action, perhaps in the form of payment or 'incentive' going well beyond the reimbursement of expenses. These incentives are particularly worrisome in the case of gamete donors (especially oocyte donors), where they may change the donor's perception of the relative risks and benefits of a donation that is not free of potential health hazards and psychological consequences. In addition, gamete donation for treatment purposes presents further ethical implications because it involves the potential generation of a new human being.

In summary, voluntary unpaid donation, long promoted as the only ethical basis for donation of bodily material, should continue to have a central role in the donation process of any type of tissue or cell. Compensation to donors should cover only justifiable expenses and loss of income, and should not act as a direct or indirect incentive or inducement.

Physicians and other health professionals should not engage in transplantation procedures, and health insurers and other payers should not cover such procedures, if the tissues or cells concerned have been obtained through exploitation or coercion of, or payment to, the donor or the next of kin of a deceased donor.

Promotion of altruistic unpaid donation of human tissues or cells by means of advertisement or public appeal may be undertaken in accordance with domestic regulations. However, advertising the need for availability of tissues or cells with a view to offering or seeking financial gain or comparable advantage for the donor, or their next of kin where the individual is deceased, should be prohibited. Brokering that involves payment to such individuals or to third parties should also be prohibited.

Tissue establishments storing and supplying human tissues and cells have developed largely in response to the increasing demand for supplies of human tissues and cells for therapy and research. However, professional bodies should ensure that their guidelines reflect their members' responsibilities in the acquisition and supply of human tissue. Tissue establishments should operate on a non-profit basis. Tissues and cells should be supplied on an operational cost basis and no payment should ever exceed the justifiable fee for the services rendered; in other words, tissue establishments can claim a reasonable amount for certain expenses but should not quote an unfair amount greater than the actual cost in order to make profits. When calculating the operational costs of a tissue establishment, which may have a variety of funding sources, consideration should be given to the long-term sustainability of the tissue establishment. In order to do this, it is important to analyse the clinical need (for the different types of tissue that the tissue establishment will be processing and distributing) and to efficiently manage the tissue establishment's 'value chain', which includes the costs of procurement, processing, storage, distribution, personnel, transport, infrastructure and administration, and the need to incorporate state-of-the-art processes and equipment, among others.

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658 The allocation of tissues and cells should be guided by clinical criteria and ethical norms, not
659 financial or other considerations. Allocation rules, defined by appropriately constituted committees,
660 should be equitable, based on clinical need, externally justified and transparent.

661 **1.7.4. Equitable access to transplantation or to medically assisted reproduction**

662 Healthcare in general is a human right because it secures and protects people's access to the
663 normal range of opportunities and because it allows people to thrive. Given the importance of health for
664 the general well-being of a person, every person, regardless of his/her income or financial means, should
665 have access to a decent minimum of healthcare.

666 Requests (the demand) for human tissues and cells may often exceed what is available (the
667 supply). Significant practical and ethical questions of efficiency and fairness arise in deciding how to
668 distribute these limited resources. Article 3 of the Additional Protocol to the Convention on Human
669 Rights and Biomedicine concerning transplantation of organs and tissues of human origin states that
670 transplantation systems must exist to provide equity in access to transplantation services for patients.
671 Except in the case of direct donations, tissues and cells must be allocated among patients only in
672 conformity with transparent, objective and duly justified rules according to medical criteria. The persons
673 or official bodies responsible for the allocation decision must be designated within this framework.

674 With regard to access to MAR, infertility treatment covers a broad range of 'causes' (e.g. age-
675 related decline in fertility, male factors, blocked Fallopian tubes) and applications that cannot necessarily
676 all be fitted into the same framework. The pivotal point in this discussion seems to be whether the desire
677 for a child should be considered a fundamental need or a personal wish. Current regulatory frameworks
678 in MAR are heterogeneous and, in some countries, still under development. The debate on ethical and
679 social issues (including access to ART for social indications, anonymity of gamete donors, genetic
680 selection of donors, compensation for donation, posthumous reproduction or surrogacy) is ongoing.
681 There is, however, general agreement that reproductive cloning must be forbidden.

682 Inclusion of infertility treatment in the basic healthcare tier is dependent upon the general level
683 of welfare in society. Cultural, educational and religious backgrounds may also affect the availability of
684 these therapies. Nevertheless, given the rightful claims of other types of healthcare and other
685 fundamental needs in society (as well a limited availability of non-partner gametes and embryos), access
686 cannot be expected to be unlimited. Ultimately, access to MAR should be considered in a structured way
687 to include efficiency, safety and equity to avoid discrimination [24]. Some countries have opted to give
688 public access to a fixed number of cycles/treatments for everyone, even if this means that those who
689 need more treatment have to pay for it themselves.

690 Cross-border reproductive care refers to a widespread phenomenon whereby patients seeking
691 MAR treatment cross international borders to obtain reproductive treatment outside their country of
692 residence. The reasons for travelling vary between countries, but the most common reason is access to
693 certain treatments or techniques not legally allowed in the country of origin (e.g. non-partner donation
694 treatment, pre-implantation genetic testing of embryos) or if a particular group is excluded from
695 treatment (e.g. same-sex couples, single women, women above a certain age). There may be other
696 limitations to access at home (e.g. long waiting lists). Further reasons for travelling may be better quality
697 of care and less expensive treatment [25, 26].

698 The ideal situation is fair access to fertility treatment at home for all patients. This ideal should
699 be promoted at all levels [26]. However, if for some reason treatment at home is not possible or not
700 available, cross-border reproductive care may provide a solution for patients. Furthermore, it is in
701 accordance with the principle of freedom of movement of patients within Europe [27]. However,
702 crossing borders may also lead to increased shortage of scarce resources in the visited country and to
703 the detriment of local citizens. Health Authorities may want to introduce a system for fair allocation of
704 scarce resources that takes into account local needs, such as a maximum number (or percentage) of
705 treatments provided to foreign patients. In addition, cross-border reproductive care should always follow
706 the same strict quality and safety criteria that govern domestic treatments, including appropriate

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707 traceability and biovigilance measures that cover both recipients and their children born as a result of
708 the MAR treatment.

709 **1.7.5. Equity in donation**

710 Individual motivation and choice is only one part of the donation picture; the central role of
711 organisations, organisational procedures and professionals in facilitating donation should not be
712 underestimated, nor indeed the importance of trust in these systems. An example of such organisational
713 aspects is that, whenever a person dies in circumstances where donation is a possibility, this should be
714 raised with their family.

715 The role of the state with respect to donation should be understood as one of stewardship: that is,
716 actively promoting measures that will improve general health (thereby reducing the demand for some
717 forms of bodily material) and facilitating donation. Such a stewardship role should extend to taking
718 action to remove inequalities that affect disadvantaged groups or individuals with respect to donation.
719 Equity in donation refers to the absence of systematic disparities in the burden of donation between
720 social groups who have different levels of underlying social advantage or disadvantage (i.e. different
721 positions in a social hierarchy). Inequities in donation would, in a systematic manner, put groups of
722 people who are already socially disadvantaged (e.g. by virtue of being poor, female and/or members of
723 a particular racial, ethnic or religious group) at further disadvantage with respect to their health.

724 As discussed above, introduction of financial incentives for donation in the field of MAR renders
725 certain social groups (and especially women) particularly susceptible to disparities based on social and
726 economic status.

727 With respect to cross-border reproductive care, safeguards must be in place to guarantee that all
728 donors, regardless their origin, receive similar care and follow-up. To prevent abuse of donors coming
729 from abroad, the use of intermediate agencies – which may lead to violations of the rules of good clinical
730 practice and, in the worst-case scenario, to trafficking – should be avoided. Post-donation care must be
731 provided to the best possible standards at home or abroad.

732 **1.7.6. Anonymity**

733 The identity of the donor and recipient should (except in the case of donation between persons
734 having a close personal relationship) be maintained in strict confidentiality. Such precautions will
735 prevent abuse and protect the families of donors and recipients from feelings of anxiety associated with
736 emotional involvement, obligation to return favours or guilt.

737 In the specific case of MAR, different regulations are applied in different member states with
738 regard to the anonymity of non-partner donors. Debate has focused around the donor's right to
739 anonymity, the welfare of the resulting offspring and his/her right to family life, and the effect of removal
740 of donor anonymity on the supply of gametes for treatment. Presently, some countries require that donors
741 always remain anonymous, whereas other countries require that their identity might be known only in
742 exceptionally urgent medical situations. Other countries allow the possibility of the offspring gaining
743 access to non-identifying information about the donor (e.g. hair colour, ethnicity). Other countries even
744 allow the offspring to contact donors after the offspring has reached a certain age. Hybrid models exist
745 in some countries. A common standard seems to be that donors do not have the right to information
746 about children generated from their gametes (unless the child chooses and is legally allowed to obtain
747 information about the donor).

748 **1.7.7. Transparency**

749 The organisation and execution of activities based on donation and human application, as well as
750 their clinical results, must be transparent and open to scrutiny, while ensuring that the personal
751 anonymity and privacy of donors and recipients are protected (if relevant).

752 Transparency can be achieved by maintaining public access to regularly updated comprehensive
753 data on processes; in particular allocation, transplant activities and outcomes for both recipients and
754 living donors, as well as data on organisation, budgets and funding. Such transparency is not inconsistent

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755 with shielding (from public access) information that could identify individual donors or recipients, while
756 still respecting the requirement of traceability. The objective of the system should be not only to
757 maximise the availability of data for scholarly study and governmental supervision to allow
758 determination of clinical outcomes and efficacy of treatments but also to identify risks (and facilitate
759 their mitigation) to minimise harm to donors and recipients.

760 1.8. Recommendations and regulations in the field

761 1.8.1. Council of Europe

762 The Council of Europe, based in Strasbourg (France), is an international organisation that
763 promotes co-operation between all European countries in the areas of human rights, democracy, rule of
764 law, culture and public health. After the 3rd Conference of European Health Ministers on the Ethical,
765 Organisational and Legislative Aspects of Organ Transplantation [28], held in Paris in 1987, the Council
766 of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ
767 Transplantation (SP-CTO) was created. This committee consisted of experts in different aspects of
768 transplantation: immunologists, surgeons and physicians, as well as co-ordinators and representatives
769 from organ-sharing and organ-procurement organisations. In 2007, the secretariat responsible for
770 activities related to organs, tissues and cells was transferred to the European Directorate for the Quality
771 of Medicines & HealthCare (EDQM) of the Council of Europe [29], and the newly appointed CD-P-TO
772 took over as the steering committee [30].
773 Today, the CD-P-TO is composed of internationally recognised experts from Council of Europe
774 member states, observer countries, the European Commission and the WHO, as well as representatives
775 from the Committee on Bioethics of the Council of Europe (DH-BIO) and several professional societies
776 and non-governmental organisations. It actively promotes the non-commercialisation of organ donation,
777 the fight against organ trafficking, the development of ethical, quality and safety standards in the field
778 of organs, tissues and cells, and the transfer of knowledge and expertise between member states and
779 organisations.
780 Within the framework principle of sharing knowledge through international co-operation, the
781 Council of Europe has established widely recognised recommendations and resolutions in the field of
782 transplantation, covering the ethical, social, scientific and training aspects of the donation and
783 transplantation of organs, tissues and cells [31]. Whereas agreements and conventions are binding on the
784 states that ratify them, resolutions and recommendations are policy statements to governments that
785 propose a common course of action to be followed.
786 The Council of Europe Convention for the Protection of Human Rights and Fundamental
787 Freedoms (European Treaty Series, No. 5) [32] is an international treaty to protect human rights and
788 fundamental freedoms in Europe. It was drafted in 1950 by the then newly formed Council of Europe
789 and came into force on 3 September 1953.
790 The European Agreement on the Exchange of Therapeutic Substances of Human Origin
791 (European Treaty Series, No. 26) [33], signed in Paris on 15 December 1958, aims to provide mutual
792 assistance with respect to the supply of therapeutic substances of human origin.
793 The European Agreement on the Exchange of Tissue-Typing Reagents (European Treaty Series,
794 No. 84) [34], signed in Strasbourg on 17 September 1974, lays the groundwork for development of
795 mutual assistance in the supply of tissue-typing reagents and the establishment of joint rules between
796 signatory parties. The signatory parties undertake to make reagents available to other parties who are in
797 need of them, by the most direct route, subject to the condition that no profit is made on them, that they
798 must be used solely for medical and scientific purposes and are free of import duties. The Additional
799 Protocol (European Treaty Series, No. 89) [35], which was opened for signature on 24 June 1976 and
800 came into force on 23 April 1977, provides for the accession of the European Community to this
801 agreement.
802 The Oviedo Convention – the Convention for the Protection of Human Rights and Dignity of the
803 Human Being with regard to the Application of Biology and Medicine (European Treaty Series, No. 164)

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[15], which was opened for signature on 4 April 1997 and came into force on 1 December 1999 – is the first legally binding international text designed to preserve human dignity, fundamental rights and freedoms, through a series of principles against the misuse of biological and medical applications. The Convention is inspired by the principle of the primacy of human beings over the sole interest of science or society. It lays down a series of principles applying to medical practice as well as biomedical research, organ transplantation and genetics. The Convention includes the principle of consent, non-discrimination on the basis of genetic characteristics, and protection of private life and access to information. The Convention specifically prohibits any financial gain from the body and its parts, as such. The Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors [22] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation.

The Oviedo Convention was extended by an Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin (European Treaty Series, No. 186) [16], which was opened for signature on 24 January 2002 in Strasbourg and came into force on 1 May 2006. This additional protocol aims to protect the dignity and identity of everyone and to guarantee, without discrimination, respect for his/her integrity and other rights and fundamental freedoms with regard to the transplantation of organs and tissues of human origin, thereby establishing principles for the protection of donors and recipients. However, the additional protocol does not apply to gametes and embryos.

The Council of Europe Convention on Action against Trafficking in Human Beings (European Treaty Series, No. 197) [36], which was opened for signature in Warsaw on 16 May 2005 and came into force on 1 February 2008, alongside its Explanatory Report, addresses the trafficking of human beings for the purpose of organ removal.

The Council of Europe/United Nations joint study on Trafficking in organs, tissues and cells and trafficking in human beings for the purpose of the removal of organs [37], presented at the United Nations headquarters in New York on 13 October 2009, focuses on trafficking in organs, tissues and cells for the purpose of transplantation. The joint study made evident that existing criminal-law instruments dealing exclusively with trafficking in human beings (including for the purpose of organ removal) left loopholes that allowed several unethical transplant-related activities to persist. This is why the Council of Europe decided to undertake the task of drafting a new international legally binding instrument against trafficking in human organs.

The Council of Europe Convention against Trafficking in Human Organs (European Treaty Series, No. 216) [36], with its Explanatory Report [36], adopted by the Committee of Ministers on 9 July 2014, identifies distinct activities that constitute 'trafficking in human organs'. The central concept is 'the illicit removal of organs', which consists of removal without the free, informed and specific consent of a living donor; removal from a deceased donor other than as authorised under domestic law; removal when, in exchange, a living donor (or a third party) has been offered or received a financial gain or comparable advantage; or removal from a deceased donor when a third party has been offered or received a financial gain or comparable advantage.

Other major resolutions and recommendations in the field of tissues and cells include:

- Resolution (78) 29 of the Committee of Ministers on Harmonisation of legislations of member states relating to removal, grafting and transplantation of human substances [17];
- Recommendation No. R (94) 1 of the Committee of Ministers to member states on human tissue banks [38];
- Recommendation No. R (98) 2 of the Committee of Ministers to member states on provision of haematopoietic progenitor cells [39];
- Recommendation Rec (2004) 8 of the Committee of Ministers to member states on autologous cord blood banks [40];

852 • Recommendation Rec (2006) 4 of the Committee of Ministers to member states on research on
853 biological materials of human origin [41].

854 Monitoring of practices in member states has become an evident need for the sake of transparency
855 and international benchmarking. Keeping this goal in mind, since 1996 the EDQM/Council of Europe
856 has published *Newsletter Transplant* [42], which is co-ordinated by the Organización Nacional de
857 Trasplantes (ONT) in Spain. This publication summarises comprehensive data (provided by national
858 focal points designated by governments) on donation and transplantation activities, management of
859 waiting lists, organ-donation refusals and authorised centres for transplantation activities. *Newsletter*
860 *Transplant* provides information from ≈70 countries, including Council of Europe member states,
861 observer countries and observer networks (e.g. Iberoamerican Donation and Network Council on Organ
862 Donation and Transplantation, Mediterranean Network). The *Newsletter Transplant* database is
863 connected with other international projects on data collection (e.g. WHO Global Observatory on Organ
864 Donation and Transplantation, Eurocet database) to avoid duplication of efforts. *Newsletter Transplant*
865 has evolved into a unique official source of information that continues to inspire policies and strategic
866 plans worldwide.
867 The Council of Europe also produces other guidelines, including this *Guide to the quality and*
868 *safety of tissues and cells for human application*, the *Guide to the quality and safety of organs for*
869 *transplantation* and the *Guide to the preparation, use and quality assurance of blood components*.

870 **1.8.2. World Health Organization**

871 In 1987, the 40th World Health Assembly, concerned about the trade for profit in human organs,
872 initiated preparation of the first WHO Guiding Principles on transplantation, endorsed by the Assembly
873 in 1991 in resolution WHA 44.25 [43]. These Guiding Principles have greatly influenced professional
874 codes and practices, as well as legislation, around the world for almost two decades. After a consultation
875 that took several years, on 21 May 2010 the 63rd World Health Assembly adopted resolution WHA 63.22
876 [44], which endorsed the updated WHO Guiding Principles on human cell, tissue and organ
877 transplantation [18] and called on WHO member states to implement these Guiding Principles, promote
878 voluntary and unremunerated donation, oppose trafficking and promote transparent and equitable
879 allocation. It also urged its members to strengthen oversight, to collect and publish activity data,
880 including adverse events and reactions, and to implement globally standardised coding. These WHO
881 guidelines are intended to provide an orderly, ethical and acceptable framework for the acquisition and
882 transplantation of human cells, tissues and organs for therapeutic purposes.
883 The World Health Assembly adopted resolution WHA 57.18 [45] in 2004, which urged WHO
884 member states "to take measures to protect the poorest and vulnerable groups from transplant tourism
885 and the sale of tissues and organs, including attention to the wider problem of international trafficking
886 in human tissues and organs". Subsequently, the Declaration of Istanbul on Organ Trafficking and
887 Transplant Tourism [19] was adopted in 2008, as an initiative of The Transplantation Society (TTS) and
888 the International Society for Nephrology (ISN). In 2018 it was updated to incorporate revised definitions
889 and briefer and more comprehensive principles to provide up-to-date guidance and practical advice for
890 health professionals, policy makers, and law-enforcement authorities. The declaration emphasises that
891 organ trafficking and transplant tourism should be prohibited because they violate the principles of
892 equity, justice and respect for human dignity, targeting impoverished and otherwise vulnerable donors
893 and inexorably leading to inequity and injustice.
894 United Nations Resolution 71/322, adopted by the WHO General Assembly on 8 September 2017,
895 aims at strengthening and promoting effective measures and international co-operation on organ
896 donation and transplantation to prevent and combat trafficking in persons for the purpose of organ
897 removal and trafficking in human organs [46].
898 Robust bi-directional donor–recipient traceability is a prerequisite to achieving effective vigilance
899 and surveillance worldwide. For this reason, Resolution WHA 63.22 [44] also urged WHO member
900 states to collaborate in collecting data (including adverse events and reactions) in addition to

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901 implementation of globally consistent coding systems. The Notify project was a specific follow-up
902 action that was led by the WHO to promote the sharing of information on adverse incidents for
903 improving safety and efficacy [47].
904 As a result of resolutions WHA 57.18 and WHA 63.22 (which requested that global data on the
905 practice, safety, quality, efficacy and epidemiology of transplantations be collected in the WHO member
906 states that have transplantation programmes), an international watchdog on transplantation was set up
907 as a collaborative initiative between the Spanish ONT and WHO, and was termed the Global
908 Observatory on Donation and Transplantation [48]. The universal availability of these data is recognised
909 as a prerequisite for global improvements in demonstrating transparency, equity and compliance, and
910 for monitoring systems in countries. In addition, the data provided also help to give an overview of the
911 legal and organisational aspects in very different settings and countries, which enables the regulating
912 bodies to monitor transplantation activities.
913 The WHO has also published two *aide-mémoires* specifically on the donation and transplantation
914 of tissues and cells [1, 49].
915 In recent years, the WHO has been promoting use of the term 'medical products of human origin'
916 (MPHO). This category includes blood, organs, tissues, bone marrow, cord blood, reproductive cells and
917 milk derived from humans for therapeutic use. Use of these MPHO, obtained from living and deceased
918 donors, entails practical, scientific and ethical considerations.

919 **1.8.3. European Union**

920 *1.8.3.1. EU tissues and cells legislation*
921 Article 168 of the Treaty on the Functioning of the European Union [50] (previously Article 152
922 of the Treaty of Amsterdam) gives the EU a mandate to establish high quality and safety standards for
923 substances of human origin, such as blood, organs, tissues and cells.
924 Acknowledging that the human application of tissues and cells is an expanding medical field that
925 offers important opportunities for the treatment of disease, the EU aims for a common approach to the
926 regulation of tissues and cells across Europe.
927 The EU tissue and cells directives have created a benchmark for the standards that must be met if
928 carrying out any activity involving tissues and cells for human applications, including gametes, embryos
929 and germinal tissue. The directives also require that systems be put in place to ensure that all the tissues
930 and cells used in human applications are traceable from donors to recipients and vice versa.
931 Directive 2004/23/EC [51] of the European Parliament and of the Council of 31 March 2004 applies
932 to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells
933 intended for human use (including reproductive cells used in ART procedures). The directive introduced
934 obligations on EU member states' authorities, from supervision of human tissue and cell procurement
935 and authorising and inspecting tissue establishments, to ensuring traceability and vigilance and
936 maintaining a publicly accessible register of national tissue establishments. It also lays down rules on
937 donor selection and evaluation (e.g. principles governing tissue and cell donation, consent, data
938 confidentiality) and quality and safety of tissues and cells (e.g. quality management, tissue and cell
939 reception, processing and storage conditions).
940 Commission Directive 2006/17/EC [52] established specific technical requirements for each step
941 in the human tissue and cell preparation process, in particular the requirements for procurement of
942 human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for
943 donors, tissue and/or cell donation, the procurement and reception procedures at tissue establishments
944 and the requirements for direct distribution to the recipient of specific tissues and cells. Directive
945 2006/17/EC was amended in 2012 by Commission Directive 2012/39/EU with regard to certain technical
946 requirements for the testing of human tissues and cells [53].
947 Commission Directive 2006/86/EC [54] includes traceability requirements, notification of serious
948 adverse reactions and events and certain technical requirements for the coding, processing, preservation,
949 storage and distribution of human tissues and cells.

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950 In 2015, two new Commission directives were adopted, one an implementing directive on the
951 procedures for verifying equivalent standards of quality and safety of imported tissues and cells
952 (Directive 2015/566) [55] and a second one amending Directive 2006/86/EC, providing detailed
953 requirements on the coding of human tissues and cells (Directive 2015/565) [56].
954 Quality and safety standards for human organs intended for transplantation are laid down in
955 Directive 2010/53/EU and Commission Implementing Directive 2012/25/EU. These detail the standards
956 and procedures for information exchange between EU member states regarding human organs intended
957 for transplantation [57, 58].
958 The EU directives dictate that EU member states must encourage voluntary and unpaid donations
959 of tissues and cells and must endeavour to ensure that the procurement of tissues and cells is carried out
960 on a non-profit basis. Promotion and publicity activities in support of the donation of human tissues and
961 cells with a view to offering or seeking financial gain or comparable advantage are not allowed. The EU
962 directives also provide clear mandates for the consent of donors and the anonymity of all data collected,
963 and instruct EU member states to adopt measures to ensure data security and prevent unauthorised
964 modifications to files and records.
965 These directives do not cover research using human tissues and cells (e.g. *in vitro* research or
966 research using animal models) and do not interfere with the decisions of EU member states on the use
967 or non-use of any specific type of human cell, including embryonic stem cells. Similarly, these directives
968 do not interfere with provisions of member states defining the legal term 'person' or 'individual'.
969 The European Commission has supported EU member states in their efforts to implement EU
970 directives on tissues and cells by providing funding for several projects under the Programme of
971 Community Action in the Field of Health [59]:

972 • EQSTB (European Quality System for Tissue Banking) focused on four main work packages:
973 (i) identification of the key requirements for tissue banking; (ii) development of a registry to
974 support exchange of tissues; (iii) provision of training programmes, both online and face-to-
975 face, to fulfil the needs of tissue establishment professionals, and (iv) development of an audit
976 model and audit guide for tissue establishments, with recommendations for tissue
977 establishments and guidance for auditors.

978 • EUSTITE (European Standards and Training in the Inspection of Tissue Establishments) [60]
979 developed guidance and training courses for EU competent authorities on the inspection of
980 tissue establishments and on vigilance procedures for tissues and cells used in transplantation
981 and in assisted reproduction. The guidance document served as a basis for the guidelines on
982 implementation of inspection and control measures in the field of human tissues and cells
983 included in Commission Decision 2010/453/EU of 3 August 2010.

984 • POSEIDON (Promoting Optimisation, Safety, Experience sharing and quality Implementation
985 for Donation Organisation and Networking in unrelated haematopoietic stem-cell
986 transplantation in Europe) provided recommendations for improvements in the safety of
987 unrelated haematopoietic progenitor cell transplantation, for the optimisation of human stem-
988 cell donation policy, and for promoting equal access to this therapy throughout the EU.

989 • EUROCET [61] is a platform that was funded initially by the European Commission but is now
990 maintained by the Italian National Transplant Centre. It collects and publishes annual activity
991 data on donation, processing and human applications of tissues and cells. However, the Tissue
992 Establishment Registry has been temporarily suspended in order to avoid confusion with the
993 official EU Tissue Establishment Compendium.

994 • EuroGTP (European Good Tissue Practices) [62] developed a guide to good tissue practices
995 and personnel training guidelines for tissue establishments on the recovery, processing and
996 preservation of tissues, to ensure that all tissue establishments guarantee the highest level of
997 quality and safety of tissues for human application. EuroGTP has provided a crucial basis for

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998 much of the technical content of this Guide. A strong collaboration between the European
999 Association of Tissue Banks (EATB), which will update and maintain the GTPs as their own
1000 standards, and the Council of Europe will be maintained to ensure consistency and development
1001 in the light of the most up-to-date scientific knowledge.

- 1002 The project SoHO V&S (Vigilance and Surveillance of Substances of Human Origin) [63]
1003 addressed the harmonisation of terminology and documentation relating to adverse events and
1004 reactions. It aimed to find a consensus on how information should be exchanged between EU
1005 member states, the European Commission and third countries to enhance efficient management
1006 of incidents involving cross-border distribution of tissues and cells. The project drafted
1007 important guidance documents for the EU competent authorities, on the detection and
1008 investigation of suspected illegal and/or fraudulent activity related to tissues and cells, the
1009 communication and investigation of serious adverse events and reactions associated with
1010 human tissues and cells, and vigilance and surveillance in the field of assisted reproductive
1011 technologies. The project also prepared a guidance document for healthcare professionals on
1012 vigilance and surveillance of human tissues and cells. It also provided a training model for
1013 competent authorities in the investigation and management of vigilance and surveillance of
1014 tissues and cells.

- 1015 The joint action ARTHIQS (Good Practice on Donation, Collection, Testing, Processing,
1016 Storage and Distribution of Gametes for Assisted Reproductive Technologies and
1017 Haematopoietic Stem Cells for Transplantation) [64], launched in 2014, was a three-year project
1018 to build institutional and inspection guidelines for assisted reproductive technologies as well as
1019 guidelines related to the set-up and regulation of haematopoietic stem-cell donor follow-up
1020 registries and banking of cord blood.

- 1021 The joint action VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted
1022 Reproduction and Transplantation) [65], launched in 2015, aimed at promoting and facilitating
1023 the harmonisation of inspection, authorisation and vigilance systems for blood transfusion and
1024 tissues and cells for human application.

- 1025 The project EuroGTP-II (Good Tissue Practices for demonstrating safety and quality through
1026 recipient follow-up) [66], launched in 2016, aimed at developing technical guidance to assess
1027 the quality and safety of novel tissue and cell therapies and demonstrating their efficacy based
1028 on recipients' outcomes.

- 1029 The joint action European Cornea and Cell Transplant Registry (ECCTR), launched in 2016,
1030 aimed to develop a common assessment methodology, based on the three existing European
1031 corneal transplant registries in the Netherlands, Sweden and the UK, and establish a web-based
1032 European registry to assess and verify the safety, quality and efficacy of ocular tissue
1033 transplantation [67].

- 1034 The EU-funded project TRANSPOSE (TRANSfusion and transplantation: PrOtection and
1035 SElection of donors) [68] was launched in 2017 and aims at harmonising European donor
1036 selection and protection policies, while maintaining adequate health and safety protection of
1037 the recipient.

- 1038 The joint action GAPP (Facilitating the Authorisation of Preparation Process for blood, tissues
1039 and cells) [69] was launched in 2018 and aims at facilitating the development of a common and
1040 optimal approach to assess and authorise preparation processes in blood and tissue
1041 establishments, adapting requirements as prescribed by Article 29 of Directive 2002/98/EC and
1042 Article 28 of Directive 2004/23/EC.

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1043 These projects have strengthened collaboration among Health Authorities, and between these 1044 Health Authorities and the professional associations in the area of tissues and cells for human 1045 application, allowing continuous input from field practice into the regulatory framework.

1046 *1.8.3.2. Other relevant EU legislation*

1047 When human tissues and cells are used in the manufacture of medicinal products that are gene-1048 therapy medicinal products or somatic-cell-therapy medicinal products or tissue-engineered products, 1049 Regulation (EC) No. 1394/2007 of the European Parliament and of the Council on advanced therapy 1050 medicinal products (the 'ATMP Regulation') applies [70, 71] (see Chapter 30). For such products derived 1051 from human tissues and cells, Directive 2004/23/EC and its implementing directives apply only to their 1052 donation, procurement and testing.

1053 Clinical trials of medicinal products containing human tissues and cells must comply with the 1054 quality and safety standards laid down in Directive 2004/23/EC and its implementing directives, in 1055 addition to the EU legislation on clinical trials [72].

1056 The EU directives on active implantable medical devices (90/385/EEC) and on medical devices 1057 (93/42/EEC) are intended to be replaced by a Regulation on Medical Devices. The final text of the 1058 regulation was agreed in mid-2016 and final formal adoption by both the Council and the Parliament 1059 was expected during the first semester of 2017. The revised requirements apply to medical devices 1060 combined with tissues and cells and medical devices incorporating non-viable derivatives of human 1061 tissues or cells, in particular human collagen [73].

1062 Directive 95/46/EC on the protection of individuals with regard to the processing of personal data 1063 and the free movement of such data [74] must be applied when processing personal data (e.g. data related 1064 to donors and recipients).

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Chapter 2: Quality management, risk management and validation

2.1. Quality management

2.1.1. Introduction

This chapter outlines the general principles of a quality management system (QMS) that should be applied at all stages, from identification of a potential donor through processing and storage of the tissues or cells to the final preparation for application to the patient. Quality of tissues and cells is achieved through compliance with requirements at four levels:

a. The legal framework that provides the overall context in which the donation, procurement, testing, processing, storage, distribution and import/export activities for tissues and cells are performed;
b. The QMS, which is a tool to ensure that tissues and cells consistently comply with technical and legal requirements;
c. The technical requirements specific to each type of tissue or cell, which ensure quality, safety and efficacy, as detailed in Part B of this Guide;
d. The authorisations in place for the specific activities, from specific competent authorities.

A tissue establishment must implement a QMS that covers the scope of all of its activities. The following non-exhaustive list of standards and legal instruments includes tools to support a tissue establishment in the construction of a robust and efficient programme:

- Good Practice Guidelines for Tissue Establishments (see Part E) that follow the EU directives. The guidelines are based on a QMS approach. They form the basis of good practice in all tissue establishments and should be used in preparation for both inspection and continuous improvement.
- The International Organization for Standardization (ISO) requirements, as addressed in the ISO 9000 QMS family of standards. ISO standards have been developed to assist organisations of all types and sizes to implement and operate effective QMS. ISO 9001 on QMS requirements is particularly relevant to tissue and cell processes.
- Good Tissue Practices for European tissue banks were developed by the EU-funded project EuroGTP, which aimed to agree harmonised practices and techniques across Europe and to increase the know-how and level of competence of tissue establishment personnel. Much of the guidance developed in that project has been incorporated in the chapters of this Guide.
- The EU Guidelines for Good Manufacturing Practices (GMP) [1] provide specific guidance for the preparation of medicinal products. However, much of their content is also relevant for the procurement, processing, storage and distribution of tissues and cells. Wherever (in the EU) products containing tissues or cells are classified as advanced therapy medicinal products (ATMP), then the full requirements of GMP must be applied.
- Directive 2004/23/EC – which sets the standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells – and its associated technical directives provide key elements to be included in a tissue establishment QMS; these requirements are legally binding in EU member states.
- FACT–JACIE International Standards for cellular therapy product collection, processing and administration, published by the Foundation for the Accreditation of Cellular Therapy and the Joint Accreditation Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation.

- NetCord-FACT International Standards for cord blood collection, processing and release for administration.
- European Society of Human Reproduction and Embryology (ESHRE) Guidelines for good practice in IVF laboratories [2].

2.2. Applying a quality management system (QMS) in donation and banking of tissues and cells

Quality is the responsibility of all personnel involved in the process of providing tissues and cells for clinical application. A systematic approach to quality management must be implemented and maintained throughout the entire process. A good system addresses quality management under the following headings:

a. Personnel and organisation;
b. Premises;
c. Equipment and materials;
d. Outsourced activities management (contractual arrangements);
e. Documentation;
f. Quality control;
g. Quarantine and release;
h. Qualification and validation;
i. Traceability;
j. Complaints;
k. Investigation and reporting of deviations, adverse events and reactions;
l. Recall;
m. Self-assessment, internal and external audit;
n. Quality risk management;
o. Fiscal and continuity planning;
p. Tools for continuous quality improvement.

2.3. Personnel and organisation

There must be sufficient, suitably qualified personnel to carry out all tasks in compliance with quality and safety requirements. Tasks and responsibilities must be clearly defined, understood and documented. All personnel should have clear, documented and up-to-date job descriptions, signed by them. There should be an organisational chart that describes the structure of the organisation with clear delineation of responsibilities and lines of reporting.

2.3.1. Key personnel

Key personnel in each organisation involved in the process (from the initial donor-selection stage to the final delivery of tissues and cells) should include an identified person who is responsible for all activities carried out in their organisation, along with a designated person who takes over this responsibility in their absence. For those countries that are members of the EU, the Responsible Person (RP) in a tissue establishment must meet qualification criteria defined in Directive 2004/23/EC. Each relevant organisation must also have an identified medical specialist/adviser who may or may not be the RP. The processing and quality-control functions should be independent to ensure the effective and reliable evaluation of processes.

The responsibilities of key personnel who manage the quality system should include (but are not limited to):

- ensuring training is completed,
- ensuring validation is completed,
- checking maintenance of premises and equipment,
- approving specifications and test methods,

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- evaluating batch manufacturing records,
- plant hygiene,
- approval and monitoring of suppliers,
- document control,
- retention of records,
- compliance of all other personnel with GMP.

Tissue and cell processing should be carried out by appropriately qualified personnel. An adequate and independent audit system should be implemented.

2.3.2. Training

Personnel must receive initial and continued training appropriate to the duties assigned to them. Criteria should be defined and satisfied before declaring personnel qualified for a specific task or processing tissue and or cells. Training methods must be documented and training records maintained. The effectiveness of training programmes should be monitored by regular assessment of the competence of personnel. Personnel should also be trained in quality principles relevant to their duties and in the broad ethical and regulatory framework in which they work. When applicable, personnel should have relevant knowledge of microbiology and hygiene, and should be constantly aware that microbial contamination of themselves, donors, recipients and tissues and cells should be avoided. The training programme should include mid- to long-term training plans, be adequately resourced and target all the personnel that might be involved in any activities within the scope of this Guide, irrespective of whether the activity is routine or occasional.

2.3.3. Safety issues for healthcare personnel working with tissues and cells for human application

Personnel carrying out tissue and cell procurement and processing activities are exposed to a risk of infection to a similar degree as operating theatre personnel. In some cases, donors will not have been fully tested at the time of procurement or initial processing and, even where they have been tested, a residual risk of infection by untested agents remains. There may also be occasions when a donation is still required to be processed following receipt of positive test results, increasing the risk to the healthcare personnel, for example when an autologous donation is assessed as being the most appropriate treatment methodology. Standard universal precautions and suitable personal protective equipment (PPE) must be applied to protect personnel from these risks. Documented procedures should be in place describing the actions to be taken if an individual is directly exposed to the blood or tissues of a donor or their donation (e.g. needle-stick injury). These procedures might include: accelerated and extended testing of the donor, rapid testing of the staff member, and prophylaxis for the transmissible agent(s) where appropriate.

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2.3.4. Safety issues for tissues or cells handled by personnel with bacterial or viral infections

Personnel involved in procurement and processing of tissues and cells might also pose a risk to the quality and safety of the tissues and cells if they themselves are infected with a transmissible agent. Organisations should have documented policies describing the requirements for health screening of personnel and for individuals to inform the organisation, in a confidential manner, if they have accidentally exposed tissues or cells to risk of contamination.

2.4. Premises,

Premises must be designed, located, constructed, adapted and maintained to suit the operations to be undertaken. Their layout and design must aim to minimise the risk of errors and permit operations to proceed in an orderly sequence. Their layout must also allow effective cleaning and maintenance to avoid contamination and cross-contamination.

Suitable, quiet premises should be available for confidential interviewing of living donors or the families or friends of deceased donors.

Facilities in which tissues or cells are procured must meet appropriate grades of air quality and cleanliness. The appropriate standard of cleanliness will depend on the type of tissues or cells being procured, the degree of exposure of the tissues or cells during the procurement process, and the decontamination or sterilisation processes that will subsequently be applied to the tissues or cells during processing. Most operating theatres are now environmentally monitored and have controlled air systems that make them suitable for the procurement of tissues that are not subsequently sterilised. Other types of facility, such as mortuaries, may also be adequate for the procurement of certain types of tissues, but they should be assessed for suitability on a case-by-case basis. Further guidance on facilities for tissue and cell procurement is given in Chapters 6, 7 and 8, and in Part B of this Guide.

Processing facilities should be dedicated to this activity and should be designed, classified, qualified, validated and monitored to ensure that air quality is appropriate for the process being carried out. An international standard should be followed in full to achieve the appropriate air quality (e.g. rules governing medicinal products in the EU, *Volume 4: EU guidelines to good manufacturing practice,* or ISO 8573-1, ISO 14644 and ISO 14698). For tissue establishments in the EU, the zone in which the tissues or cells are exposed to the air must be equivalent to Grade A, with a surrounding environment of at least Grade D (GMP classification), unless specifically defined criteria for exemption from this requirement are met; the latter applies notably in the field of assisted reproductive technology (ART). Some national requirements are more stringent, requiring Grade B and C backgrounds for certain processes or tissue or cell types. Processing and storage facilities should be cleaned according to a schedule and procedure that has been validated to achieve the required level of cleanliness and all cleaning procedures should be documented. Where products containing tissues and cells are classified as ATMPs in the EU, fully GMP-compliant facilities are required.

More specific guidance on requirements for processing facilities is given in Chapter 8 and in Part B of this Guide.

Storage conditions for tissues and cells must be controlled and monitored. If certain conditions are critical to maintenance of the required properties of tissues or cells, appropriate alarms must be in place to indicate if conditions are approaching, or fall outside, predefined limits. Standard operating procedures (SOPs) should define the actions to be taken in response to alarms. Storage requirements apply equally to interim storage of tissues and cells before transport to a processing facility. Further guidance on requirements for storage is given in Chapter 9 and in Part B of this Guide.

Premises should include adequate dedicated areas that allow the ‘first in, first out’ – or, when applicable, the ‘first expired, first out’ – principle for critical consumables and reagents to be respected. In this context, ‘critical’ means those consumables and reagents that come in contact with the tissues or cells or influence the critical quality/safety attributes of the tissues and cells directly (e.g. an additive) or indirectly (e.g. donor testing kits). These areas should allow for adequate (physical or electronic) segregation of those materials in quarantine from those released for use. They should be temperature-mapped and monitored when necessary.

2.5. Equipment and materials

2.5.1. Equipment

A list or register of equipment that might influence the quality or safety of the tissues or cells should be maintained (a validation plan). All equipment on this list must be designed, qualified, validated and maintained to suit its intended purpose and all such equipment must minimise any hazard to donors, recipients, operators or the quality and safety of the tissues and cells. The validation plan should be designed through a risk-assessment exercise and should indicate when and how critical pieces of equipment should be validated and re-validated as necessary (see §2.16). Equipment should be selected that permits effective cleaning. Maintenance, monitoring and cleaning must also be carried out according to a schedule and documented in equipment logbooks.

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Trending and analyses of calibration and monitoring results (e.g. via statistical process control) should be a continuous process. Intervals of calibration and monitoring should be determined for each item of equipment to achieve and maintain a desired level of accuracy and quality. The calibration status of all equipment that requires calibration must be readily available.

To ensure appropriate performance of a system or equipment, a monitoring plan must be developed and implemented. The plan should take into account the criticality of the system or equipment, and should outline monitoring, user notification and mechanisms for problem resolution. If an unusual event is observed, personnel should follow the standard response described in the monitoring plan. The standard response should involve notifying affected personnel and, if possible, initiation of a resolution response to the problem and risk assessment of the affected tissues or cells. Depending on the severity of the problem and the criticality of the system or piece of equipment, a back-up plan may need to be implemented to keep the process or system operating.

All equipment with a critical measurement function must be calibrated according to a planned schedule. Calibration is a procedure that confirms, under defined conditions, the relationship between values obtained from an instrument or system and those obtained using an appropriate certified standard. Calibration addresses accuracy and precision. 'Measurement accuracy' refers to the closeness of agreement between a measured quantity value and the true quantity value of what is being measured. 'Measurement precision' refers to the closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Hence, if the measured value is close to the true value, the measuring system has high accuracy and if the spread of the values is small when measurements are repeated, the measuring system has high precision. The acceptable tolerance should be set according to the critical quality attributes of a tissue/cell and these tolerance limits of the same equipment may have different needs depending on the tissues/cells subject of the process.

In practice, each piece of critical measuring equipment must be traceable. There must be an unbroken chain of calibration back to a recognised standard. Hence, the equipment is compared against a standard; the standard is compared against a higher standard; and the chain is documented through calibration certificates. If calibration is carried out by a third party, a copy of the calibration certificate for the specific measuring device used must be provided together with the calibration report. There must be an SOP that provides specific requirements for the calibration of each measuring device, such as defining the frequency of calibration, the number of measurement repeats, and the expectations and interpretation of obtained results which define acceptable limits for accuracy and precision. If the limits of accuracy and precision are not met, there must be provisions for remedial action to re-establish conformity with these limits. These remedial activities must be documented. If calibration activities provide evidence that tissues or cells were processed and released for use when critical measurement equipment was not measuring accurately or precisely, risk assessment should be applied to decide on appropriate corrective or preventive actions regarding the fate of the tissues and cells.

A periodic review process should be established to ensure that the documentation for system or equipment is complete, current and accurate. If deviations or problems are found, actions should be identified, prioritised and planned.

2.5.2. Materials, consumables and reagents

A controlled list should be constructed of all materials and consumables that come into contact with the tissues or cells or that influence the quality or safety of the tissues or cells. Detailed specifications for these critical reagents and consumables must be documented. Only materials from qualified suppliers that meet the documented specifications should be used. When indicated, manufacturers should provide a certificate of compliance for every lot/batch of materials supplied. Batch acceptance testing or checking of each delivery of materials should be carried out and documented before release for use in tissue or cell procurement or processing. Equipment and materials should conform to international standards and EU and national licensing arrangements, where these exist.

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1483 Inventory records must be kept for traceability and to prevent use of materials after their expiry
1484 date. Each batch of critical reagents or consumables must be traceable to the respective procurement or
1485 processing session of tissues and cells in which they were used.
1486 Apparent deviations in the quality and/or performance of equipment and materials must be
1487 investigated and documented promptly. Outcomes of these investigations should be reported in a timely
1488 manner to the RP, who should consider and approve the corrective and preventive actions to be
1489 implemented. For relevant deviations, a notice should be sent to the manufacturer and, where
1490 appropriate, reported to the Health Authority.
1491 Further guidance on reagents and materials used in tissue and cell processing is provided in
1492 Chapter 8.

1493 2.6. Outsourced activities management (contractual arrangements)

1494 Where steps influencing the quality or safety of tissues or cells (i.e. critical steps) are carried out by a
1495 third party, there must be a contract or service-level agreement in place that describes the roles and
1496 responsibilities of all parties for maintaining the quality chain and the quality requirements for the
1497 service provided. Agreements should allow for on-site audits of contracted third parties to confirm their
1498 compliance with expectations. An example of an expectation is that if a supplier changes specifications
1499 for equipment or reagents provided to a tissue establishment, or they provide a substitute for an ordered
1500 item, they must first ensure that these changes are acceptable to the tissue establishment.
1501 In EU member states, tissue establishments must establish written agreements with a third party
1502 each time an external activity takes place that influences the quality and safety of tissues and cells
1503 processed in co-operation with a third party. They must keep a complete list of these agreements and
1504 make them available at the request of Competent Authorities.
1505 Agreements must be dated, reviewed and renewed on a regular basis. Written agreements should
1506 be in place for at least the following service suppliers:
1507 a. testing laboratories (including donor, tissue and environmental testing);
1508 b. procurement teams that are independent from a tissue establishment;
1509 c. transport companies;
1510 d. suppliers of critical equipment, consumables and reagents;
1511 e. suppliers of services such as tissue and cell storage, processing or sterilisation;
1512 f. suppliers of software applications.

1513 Once tissues and cells have been distributed for clinical application, they usually leave the QMS
1514 of the tissue or cell facility. However, appropriate control of transport and storage conditions,
1515 appropriate handling and preparation before use, and full traceability must all be maintained.
1516 Maintenance of quality and traceability is usually achieved by providing users with clear and detailed
1517 written instructions. The establishment should implement appropriate measures, such as instructions for
1518 use and traceability record labels, which are provided to the clinical user in order to ensure product
1519 quality and traceability down to the recipient. Some national standards require the organisation
1520 responsible for human application of tissues and cells (ORHA) to provide the supplying tissue
1521 establishment with details of the patient to whom the tissues or cells were clinically applied. However,
1522 this is not necessary for partner donation treatment in an ART centre or autologous haematopoietic
1523 progenitor cell transplantation where all steps (including clinical application) are carried out in the same
1524 facility.
1525 Distribution direct to the patient for use without supervision of a health professional (e.g. serum
1526 drops) requires particular attention to instructions for storage and use, and should be carried out only if
1527 it is the only available option. Direct distribution of sperm to individuals for use without supervision of
1528 a health professional must be avoided for quality and safety reasons.

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2.7. Documentation

Documentation must enable all steps and all data relating to the quality and safety of the tissues and cells to be checked and traced, from the donor to the recipient and vice versa (see Chapter 15, Traceability). In ART, traceability also involves follow-up of the outcome from these treatments, including the children (see Chapter 27). Written documentation ensures that work is standardised and prevents errors that may result from oral communication. Where oral communication is necessary for critical information exchange, audio recordings may be useful. Donor documentation in general, and donor-referral records in particular, must be subject to the same controls.

Documentation must be version-controlled and include at least the following items:

a. a quality manual;
b. specifications for materials and reagents;
c. approved SOPs for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;
d. identification and analysis of risks and a risk-mitigation plan;
e. records of the performance of operations, including processing records;
f. records of complaints, audits and non-compliances;
g. training and competency records of personnel;
h. qualitative and quantitative specifications for tissues and cells;
i. key quality indicators for tissues and cells.

Documents, including SOPs and forms, must be approved by appropriate and authorised persons and be part of a document-control system that ensures that only the current version of the document is in use. The system for distribution of controlled documents must ensure that all relevant personnel have access to the correct version.

A documented system for change control should be in place that controls changes to premises, equipment, processes, personnel and any item that may impact the quality and safety of the tissues and cells. This change-control system should link the rationale for change with the approval/rejection of the proposed change, criticality of the change with respect to the quality and safety of the tissues and cells; impact of the change on the tissue establishment as a whole, validation requirements of the proposed change and associated training requirements.

Records must be legible and indelible and should not be handwritten, except for those situations where data can be recorded only in this way. Any alterations made to a record must be dated and signed or in the case of digital records an audit trail of alterations must be recorded. Documentation must be retained according to national requirements. Processing records must be maintained for all critical steps, and they must be dated and signed by the personnel responsible for carrying out the activity. All quality-control tests and checks must be documented. Any deviations from the standard documented procedures must be recorded and reviewed, and corrective action must be documented.

The QMS must define the period of time for which documents will be retained. In the EU, records that are critical for the safety and quality of tissues and cells, including quality-system documentation and raw data, must be retained for 10 years and traceability documentation for 30 years after use or expiry of the tissues and cells.

Data can be stored on paper, electronically or on microfilm. An establishment responsible for using personal data has to follow strict rules on data protection. They must make sure the information is:

- used fairly, lawfully and transparently,
- used for specified, explicit purposes,
- used in a way that is adequate, relevant and limited to only what is necessary,
- accurate and, where necessary, kept up to date,
- kept for no longer than is necessary,
- handled in a way that ensures appropriate security, including protection against unlawful or unauthorised processing, access, loss, destruction or damage.

1579 Information which is deemed more sensitive may require additional protection. This includes data
1580 on:
1581 • race,
1582 • genetics,
1583 • biometrics (where used for identification),
1584 • health.

1585 International and national regulations on data protection have to be adhered to. See Chapter 13,
1586 Computerised systems, for further information on data protection. Personnel should have access only to
1587 those categories of data for which they are authorised.
1588 Quality specifications should be prepared for each type of tissue and cell graft; these should be
1589 the basis for quality-control testing and product release.

1590 2.8. Quality control

1591 'Quality control' refers to those activities, such as verification steps, sampling and testing, which are
1592 used to ensure that materials, processes and the final product meet the required specifications. Internal
1593 quality control in a testing laboratory includes use of positive, weakly positive or negative control
1594 samples as appropriate. External quality assessment (sometimes called 'proficiency testing') involves
1595 analysis of unknown samples and evaluation of the results by a third party. Quality control of critical
1596 functions can be undertaken using audit techniques that include a sampling plan.
1597 Detailed guidance on microbiological testing is provided in Chapter 10. Guidance on specific
1598 quality-control tests for specific types of tissues and cells is provided in Part B of this Guide.

1599 2.9. Quarantine and release

1600 All tissues and cells must be stored with an unambiguous quarantine status until all quality-control tests
1601 and checks have been conducted and the results reviewed by the individual responsible for release.
1602 Release of tissues and cells may be conducted in two steps. The first step confirms compliance of the
1603 donor with defined acceptance criteria (which is usually carried out by clinical personnel). The second
1604 step confirms compliance of the tissues or cells themselves, their characteristics, processing and storage,
1605 with those criteria defined in the product specification. The latter is usually carried out by quality-
1606 assurance personnel. The concept of 'quarantine and release' is not applicable to partner donation in
1607 ART and to some types of autologous or direct donation (see §12.5 for guidance on exceptional release).
1608 Tissues and cells that cannot be categorised as 'released' during storage must be stored with an
1609 unambiguous quarantine status.
1610 For further information, see Chapter 9, 'Storage and release'.

1611 2.10. Change control

1612 Change-control procedures should ensure that sufficient supporting data are generated to demonstrate
1613 that the revised process results in a product of the desired quality consistent with the approved
1614 specifications. Change control should be carried out prior to the implementation of a revised/new
1615 process. Written procedures should be in place to describe the actions to be taken if a change is proposed
1616 to any starting material, final product specification, equipment, environment (or site), method of
1617 production or testing, or any other change that may affect the quality of tissues or cells or the
1618 reproducibility of the process. All such changes should be requested, documented and accepted formally.
1619 The likely impact of the change in facilities, systems and equipment on the final product should be
1620 evaluated (including a risk analysis). The need for, and the extent of, any re-validation should be
1621 determined.
1622 The training programme should be re-assessed for any critical change in environment, equipment
1623 or processes. Training records (including plans and training plans) must ensure that training needs are

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identified, planned, delivered and documented appropriately by taking into account any changes to systems and equipment.

Some changes may require notification to, or licence amendment from, a national regulatory authority.

2.11. Traceability

Full traceability – both of donations from donor to recipient and of all materials, reagents and equipment that come into contact with tissues and cells – is fundamental to recipient safety. Detailed guidance is provided in Chapter 15.

2.12. Complaints

All complaints must be documented, carefully investigated and managed in a timely manner. The complaints procedure should take into consideration complaints from:

a. living donors or the families of deceased donors;
b. personnel;
c. third-party health professionals;
d. clinical users, including those in another jurisdiction;
e. patients.

A mechanism for categorising, tracking and trending complaints should be in place and should be readily available for audit. Categorisation of complaints should in any case lead to the assessment of whether the complaint is justified and related to a potential non-compliance. Any potential non-compliance should then be investigated thoroughly, including root cause analysis and identification of corrective measures (see §2.13, §2.18 for details).

2.13. Investigation and reporting of deviations, adverse events and adverse reactions

—A deviation (which could be understood as non-conformity) might result in an adverse reaction in a living donor or in a recipient and must, therefore, be linked to the vigilance reporting system. There must be an SOP in place that defines how the organisation manages deviations and this SOP must include a log of all the instances of deviations that are investigated, including detailed documentation of the investigation, root cause analysis and corrective/preventive actions taken. A categorisation of deviations, depending on how critical they are to the quality and safety of tissues and cells, is a useful tool for prioritising corrective actions.

Procedures should be in place to identify appropriate corrective and preventive actions to be taken and to inform the relevant authorities as appropriate. Reporting of errors and incidents in a non-punitive context should be encouraged to help achieve improvements in practice. Tracking and trending of deviations should be carried out to identify common failures and identify areas for concern.

Serious adverse events and serious adverse reactions should be reported through a vigilance system. For detailed guidance on vigilance of tissues and cells, see Chapter 16. If products containing tissues or cells are classified in the EU as ATMPs, adverse occurrences should be reported either through pharmacovigilance systems for process events or through biovigilance systems for donor reactions.

2.14. Recall

An effective written procedure must be in place for recalling defective tissues or cells or those suspected of not meeting required quality or safety requirements. This written procedure must encompass the need to agree and document any corrective and preventive actions that might be necessary, remembering that other tissues or cells procured from the same donor might be affected. Therefore, a recall procedure could affect more recipients than initially presumed. If other organs from the same donor were used,

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transplant teams should be promptly informed. The actions should be communicated to the end user, where appropriate. Further guidance on recall is provided in Chapter 16.

2.15. Self-assessment, internal audit and external audit

Auditing is an essential tool for ensuring compliance with the quality system and for supporting continuous quality improvement.

Internal audits should be scheduled and conducted in an independent way by designated, trained and competent persons. Internal audits are normally carried out by the organisation's quality assurance personnel.

External audits are undertaken by independent bodies (often designated as approved/competent authorities or ISO certifying bodies) and are required for certification, accreditation and licensing purposes. External audits provide an opportunity for critical review by experts unfamiliar with the systems in place locally. They can provide an excellent opportunity for systems improvement.

All audits should be documented and recorded. Clear procedures are required to ensure that the agreed corrective and preventive actions are undertaken appropriately. These actions and their completion should be recorded.

2.16. Qualification and validation

2.16.1. General principles

Validation is the part of the QMS concerned with proving that all critical aspects of the establishment's operations are sufficiently under control to provide continual assurance that tissues and cells will remain safe for patients and fit for purpose. The critical aspects subject to validation include:

a. the facilities and equipment used in procurement, processing, storage, testing and distribution, and any software used to manage their operation and data;
b. materials and reagents used which come into contact with cells and tissues;
c. labelling and tracking materials, equipment and software;
d. operational staff and the written procedures that they use to instruct their work;
e. process stages from procurement to distribution where there is a risk of a detrimental effect on the quality and safety characteristics of tissues and cells if not performed correctly;
f. analytical test methods used to assess and confirm the safety and quality of donors, donations, tissues and cells;
g. other auxiliary processes such as the transport and cleaning processes.

Validation is a highly technical activity requiring a good understanding of the risks associated with critical processes conducted by the tissue establishment and the potential risks and impact of materials and equipment used in these processes. Small establishments performing very simple, minimal manipulation of a limited range of tissues and cells in accordance with published methods, or following long-established practices using the same materials and equipment, may rely on ongoing quality control and periodic reviews to confirm that the method has the intended outcome. Such small establishments should still document their validation policy, explaining their approach on the basis of risk.

The risks increase significantly with the introduction of more complex processes, a wider range of tissue and cells handled, computerised systems, expansion of facilities and significant growth in workforce. In these circumstances it becomes more important to take the formal approach to validation as described in this section to ensure that the establishment's processes remain safe for donors and patients.

Validation is usually split into two components, qualification and process or test-method validation. The term 'qualification' is applied to each part of the process and to individual items including cleanroom facilities, equipment, computer systems, materials and operators. Such items should be qualified before they are first used in a process and then re-qualified at predetermined

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intervals, or when significant changes are made. Each individual item should be qualified separately to demonstrate consistent performance.

Process (or test-method) validation should only be performed once all the items used have been qualified. Process validation should be performed before a new process or method is used routinely and, where required by local legislation, before any associated tissue or cell product is released for human application (prospective validation). It is possible to conduct process validation during the processing of tissues and cells intended for subsequent release for human application (concurrent validation). Where establishments have not validated any of their processes or methods because they have been in routine use without change for many years, they may use existing data and information as a basis for validation (retrospective validation). Any process or method changes should be assessed for impact and risk in accordance with quality risk-management principles (see §2.16) and re-validation should be considered where there is unacceptable risk.

The objective of validation is to challenge the critical aspects of items through a series of controlled tests representative of the conditions under which they are expected to operate, to demonstrate that they achieve predefined acceptance criteria for quality and safety. The challenge should include the normal variation of possible conditions expected, but also more extreme conditions where there are high risks, to provide a safety margin, for example with sterilisation processes. The test methods to be used and the acceptance criteria should be documented and approved by the establishment management before qualification or process validation commences. This document is commonly called the validation plan. The validation should be performed by trained and competent persons. The results of the validation should be compared with the acceptance criteria and any deviation from the plan should be recorded during the validation and documented in summary form with a conclusion. This document is commonly called the validation report. Following validation, the acceptance or rejection of the item or process by designated establishment management should be documented.

It should be clear through documentation and/or status labelling which processes and items have been validated and which are in the process of validation. Where anything is not in a fully validated state, there must be controls to prevent its use.

2.16.2. Validation planning

The validation policy should consider a process design phase where deep knowledge of the process is achieved. In this stage, the critical quality attributes of the tissues/cells are identified and the subsequent critical process parameters are identified. According to the critical process parameters affecting the critical quality attributes, a process control strategy should be developed.

During the validation phase itself, the process control strategy is implemented, and all the elements involved, such as equipment, utilities, suppliers and transport, are qualified before proceeding with the process validation.

All validation must be carefully planned in advance. Validation planning requires technical expertise in the processes involved and items used in the processes as well as expertise in any applicable regulations and technical and quality standards. It should therefore involve a validation team of relevant operational, quality, regulatory and medical experts in the establishment. Where necessary, for example the validation of new cleanroom facilities, external experts may be employed to advise.

Effective validation is not possible unless the establishment management is completely clear what its specific technical and quality requirements are. Establishments should use quality risk-management procedures to help determine their expectations for the processes and items used, which must address any significant risks to donors, recipients or quality of products, and any risks of regulatory non-compliance. Consideration should be given to possible modes of failure and the need to detect failure. Each establishment should document their expectations in the form of specifications. For processing stages there should be product specifications, for test methods there should be test specifications and for items used in processes, there should be user-requirement specifications (URS). For bespoke items of equipment and facilities, the manufacturer or supplier will need to interpret the URS and write a design specification to instruct their engineers, who will then write associated detailed technical specifications

for the construction. For off-the-shelf items, the URS is used to assess suitability and to inform purchase decisions.

The validation team will need to develop validation plans that prove that the relevant specifications will be met under all likely conditions, with expected margins of safety where necessary. Appropriate tests and associated acceptance criteria should be established. This requires knowledge of the critical operational parameters and the expected variation in those parameters. Such parameters may include operating temperatures, exposure times, air flows, bioburden, location and product characteristics. Statistical methods will often be needed to demonstrate consistent performance with the necessary level of confidence. Means for detecting failures and alarms will need to be tested. Where relevant standards such as ISO and the *European Pharmacopoeia* provide detailed validation methods, these should be included in the validation plan.

If it is not possible to complete any procedures strictly in compliance with the plan, then there should be a review by the validation team, who should decide whether to repeat all or part of the validation or to allow a deviation for the modified procedure. Such decisions must be recorded in the validation records and report. Where establishments employ an independent quality manager or a designated RP in accordance with EU legislation, then that person should supervise all validation activity and be responsible for approving or rejecting the outcome of validation.

2.16.3. Documentation

As with all elements of the quality system, the policy and process for planning, executing and recording validation must be documented in written procedures. This documentation may be assembled into a validation master plan (VMP). The VMP will typically include the following:

a. validation policy;
b. organisational structure of validation activities;
c. summary of facilities, systems, equipment and processes to be validated;
d. documentation format;
e. planning and scheduling;
f. change control;
g. reference to existing documents.

2.16.4. Qualification of operators

Operators are qualified as part of the training programme (see §2.3.2). There should be a documented training specification and plan for each operator, identifying how they are to be trained and listing the desired outcomes (acceptance criteria) from the training. The capability of individual operators to meet the desired outcomes should be assessed through observation and tests before they are approved as qualified to carry out procedures unsupervised. Particular attention is given to qualification of operators' aseptic techniques. The usual approach is to conduct simulated processes using culture medium or broth in place of, or added to, tissues or cells. Each operator should be qualified by performing, for example, three consecutive medium-simulation processes.

Before written procedures (SOPs and Work Instructions) are approved as part of the document-control system (see §2.7) they should be read and qualified by an experienced operator to confirm that they are clear, understandable, accurate and practical.

2.16.5. Qualification of materials and suppliers

Detailed User Requirements Specifications (URS) should be available for materials (see §2.5.2 and §2.16). Before introducing a new material into a process it must be qualified. This will involve confirmation that it meets the URS through examination of the material and of test data either from the manufacturer (Certificate of Analysis), a third party or in-house testing. Where the URS requires, or where there may be significant variation that might affect the outcome from the process in which the material is to be used, there may be a need for process qualification of the material. Process qualification

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may be carried out on a scaled-down version of the process or at full scale and may be run in parallel with the existing material to demonstrate a comparable outcome.

Suppliers and manufacturers of materials, equipment and contract services should be qualified before any purchase is made. The purpose is to ensure that they can be relied upon to continue supplying the goods and services that meet the URS. This relies upon compliance with quality specifications and operation of an acceptable quality system. This should be confirmed through audit (see §2.15) of their operations and quality system. It is possible to conduct the audit through a questionnaire supported by copies of relevant certification from a recognised independent body or regulatory authority. Otherwise an on-site audit should be performed. The information gathered through the audit should be formally documented and assessed before the supplier is considered qualified.

2.16.6. Qualification of facilities and equipment

Facilities and equipment should be qualified and processes must be validated before use and when any significant change is implemented.

Facilities and equipment should be qualified following the four steps shown in Figure 2.1. Each step should be completed, and verification obtained that acceptance criteria have been met, before proceeding to the next step.

Figure 2.1. Steps in qualification of facilities and equipment

2.16.6.1. Design qualification

The first element of the validation of new facilities, systems or equipment can be considered ‘design qualification’ (DQ). This validation involves demonstration and documentation of the compliance of the design with good practice (i.e. the design is suitable for the intended purpose). DQ is not required for off-the-shelf equipment and systems as these have already been designed and built for specific uses. Only new facilities and equipment being designed or built specifically for the establishment require DQ, which should be complete before fabrication of equipment, systems or facilities starts.

2.16.6.2. Installation qualification

Installation qualification (IQ) should be carried out on new or modified facilities, systems and equipment once installed on site. IQ should include (but is not limited to) the following:

a. installations of equipment, piping, services and instrumentation, which are checked to current engineering drawings and specifications;
b. collection and collation of the operating and working instructions as well as the maintenance requirements of the supplier;
c. calibration requirements, including verification of the uncertainty of measurement for any measuring equipment;
d. verification of construction materials.

IQ for new facilities and more complex equipment may be performed by the supplier, but the establishment should verify that agreed acceptance criteria have been met. An example of a cleanroom qualification document is available in **Appendix 4**.

2.16.6.3. Operational qualification

Operational qualification (OQ) should follow IQ. OQ should include (but is not limited to) the following:

a. tests that have been developed from knowledge of processes, systems and equipment;
b. tests to include a condition or a set of conditions encompassing upper and lower operating limits (sometimes referred to as ‘worst-case’ conditions).

Completion of a successful OQ should allow calibration, operating and cleaning procedures, operator training and preventive maintenance requirements to be finalised. It should permit a formal ‘release’ of the facilities, systems and equipment.

2.16.6.4. Performance qualification

Performance qualification (PQ) should follow successful completion of IQ and OQ. PQ should include (but is not limited to) the following:

a. tests, using production materials, qualified substitutes or simulated products, which have been developed from knowledge of the process and the facilities, systems or equipment;
b. tests to include a condition or set of conditions encompassing upper and lower operating limits.

The number of tests carried out should achieve reproducibility of the process, to the grade of warranty that the process is required to demonstrate. The more knowledge there is of the process, the less testing during PQ may be needed.

2.16.7. Qualification of software

Computer and automated systems controlled by bespoke and off-the-shelf software are extensively used by tissue and cell establishments to help manage procurement, processing, testing and distribution activities and data. The design of, and changes to, software can have a significant impact on the quality and safety of tissues and cells and the integrity of critical data. It is therefore essential to maintain effective version control over software in use and to qualify new software and requalify existing software when changes are made. Establishments should be aware of the current version of software operating their relevant computer and automated systems at all times and should not allow upgrades of existing software by system suppliers without their approval.

Software requirements should be included in the appropriate URS for the system they control. For bespoke software, the URS will be used by software system engineers to produce a detailed technical specification to be used by a programmer to write new or revised code for the system. For an off-the-shelf system the URS will be used to assess candidate software and to inform the local IT staff how to configure the software. New and revised versions of software should be qualified in operation by users (user acceptance testing or UAT) before being put into service. At the very least, this testing should include verification of critical settings encoded in the software, for example when there is a version update to software for running an apheresis machine or testing system. However, usually the software will be tested in its operational state through process-simulation tests or parallel running as part of the system (equipment) qualification. It is important during user acceptance testing to verify that existing functionality continues to operate as expected (regression testing) as well as testing new functionality.

For additional information, see Chapter 13.

2.16.8. Test-method validation

The approach to test-method validation will depend on whether the test is quantitative or qualitative. In all cases the validation plan should take into account the variety of sample types and analytes to be tested, as there may be substances present that interfere. For quantitative assays, the

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1895 acceptance criteria should consider accuracy, reproducibility, linearity, limits of detection and required
1896 range of measurement. The uncertainty of measurement should be established and quoted with
1897 subsequent results. For qualitative tests, then specificity and sensitivity are the key criteria. It may be
1898 considered unnecessary to perform in-house method validation if test systems and kits certified as
1899 compliant with the EU *In vitro* Diagnostic Medical Device Directive are used along with qualified
1900 equipment in accordance with the manufacturer's instructions. However, in-house verification studies
1901 should be done to demonstrate that the performance of the kit or test system, as used in the establishment,
1902 meets the expected specification. If using *Pharmacopoeia* methods, e.g. for sterility testing, the methods
1903 must be validated in accordance with the method monograph.

1904 **2.16.9. Process validation**

1905 Process risk-assessment methods should be used to identify what processing stages require
1906 validation. Most processing of tissues and cells involves the removal, exclusion or reduction of
1907 unwanted or undesirable substances, while maintaining the functionality of the required tissue or cells.
1908 As a minimum, validation will focus on demonstrating that the desired characteristics are achieved in
1909 performing consecutive processes (usually three) and confirming that the purity specifications have been
1910 reproducibly met in all cases. Process validation may also include *in vitro* and *in vivo* tests of
1911 functionality, where there is a risk that this may be affected. However, because of the inherent variability
1912 of human cells and tissues, establishments should consider supplementing prospective or concurrent
1913 validation with an ongoing process-verification programme of quality-control testing before release and
1914 of quality monitoring.

1915 The processes for removing undesirable substances and in particular potentially pathogenic
1916 micro-organisms should be validated with a safety margin, or 'worse case' scenario. This will usually
1917 involve spiking the material with a larger-than-normal level of the undesirable substance and
1918 demonstrating its effective removal, or reduction to safe levels, by the process. In the case of micro-
1919 organisms, strains that are known to be resistant to antimicrobial treatment, e.g. spore-forming, heat-
1920 resistant bacteria, may be used for spiking and to validate sterilisation processes, at sufficient levels to
1921 demonstrate at least a 6-log reduction. For safety-critical process validation it is recommended that
1922 published guideline methods are consulted where available.

1923 More specific guidance on approaches to validation is given in other sections of this Guide. Some
1924 examples of qualification are given in Appendices 4 and 5, and examples of validation are in
1925 Appendices 6 and 7.

1926 2.17. Risk management

1927 The procurement, testing, processing, storage and distribution of tissues and cells should be subjected
1928 to comprehensive risk assessment to allow identification of those steps where most of the quality-system
1929 controls are required and where validation of procedures is necessary. A 'process flow' diagram listing
1930 all relevant steps, processes, reagents, tests and equipment can form the basis for the assessment
1931 exercise. Risk assessment should include an estimation of the severity of any identified hazard (source
1932 of harm) and an estimation of the probability that the hazard will result in harm. Probability should be
1933 based on evidence and experience whenever possible.

1934 Risk-mitigation strategies should be developed to protect the tissues and cells, the donor and
1935 recipient, personnel and the process itself, as well as other processes being undertaken in proximity to
1936 it. The degree of control within the quality system should be related to the degree of risk associated with
1937 each step in the process.

1938 Risk assessment should refer to current scientific knowledge, should involve appropriate technical
1939 expertise and should be related to the protection of the patient. The level of effort, standardisation and
1940 documentation of the risk-control process should be aligned with the estimated risk level.

1941 Risk assessment should be repeated and documented whenever a critical process is changed as
1942 part of a change-control process. Actions to mitigate any significant new risks, including validation,
1943 should be completed before any change is implemented.

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1944 Risk assessment is also an essential tool for making important decisions, particularly when
1945 departures from standard procedures or their standards and specifications are under consideration.
1946 Examples would include:
1947 a. selection of a donor where full compliance with the normal criteria has not been met, but where
1948 the donation has a particular clinical value and the potential risk can be mitigated sufficiently to
1949 justify the deviation from standard procedures;
1950 b. exceptional release of non-complying tissues or cells on the basis that the potential benefits for
1951 the recipient and the lack of availability of alternatives outweigh the potential risks;
1952 c. retention or removal of tissues and cells in storage that had been historically released according
1953 to former criteria, when new, more sensitive procedures or tests have been implemented that imply
1954 an additional level of safety or quality and new, more stringent criteria for release;
1955 d. eligibility determination where certain test results are reactive, for example, where EU Directive
1956 2006/17/EC Annex 2 requires further investigations with a risk assessment when antibody to
1957 hepatitis B core antigen (anti-HBc) is positive and hepatitis B surface antigen (HBsAg) is negative
1958 or where a donor is reactive for a *Treponema*-specific test (see Chapter 5 for further guidance on
1959 donor testing);
1960 e. prioritisation of a list of corrective actions following an audit or inspection, or prioritisation of
1961 quality improvements in general.

1962 The approach to risk assessment should be systematic and should be documented. The most
1963 commonly applied risk-assessment methods are Hazard Analysis and Critical Control Points (HACCP),
1964 Failure Mode and Effects Analysis (FMEA) and Failure Mode, Effects and Criticality Analysis
1965 (FMECA).

1966 *2.17.1. Hazard Analysis and Critical Control Points*

1967 HACCP was developed in the 1950s in the food industry but is now widely used for many manufacturing
1968 processes, including biological control. HACCP is also recognised by ISO 14644 as a formal system for
1969 risk assessment. HACCP is a system that requires that potential hazards are identified and controlled at
1970 specific points in a process. HACCP has seven principles:
1971 1. Conduct a Hazard Analysis (HA).

1972 2. Identify the Critical Control Points (CCP).

1973 3. Establish the critical limits.

1974 4. Monitor CCP.

1975 5. Establish corrective actions.

1976 6. Verification.

1977 7. Record keeping.

1978 The World Health Organization published a paper in 2003 providing more details on using
1979 HACCP as a tool within a pharmaceutical environment, details which can be further adapted and
1980 interpreted for use within a tissue establishment [3].
1981 The *Quality Assurance Journal* has also published papers on the use of HACCP as a tool within
1982 a QMS [4].

1983 *2.17.2. Failure Mode, Effects and Criticality Analysis*
1984 FMECA is an extension of Failure Mode and Effects Analysis (FMEA) that includes a factor for
1985 detectability, taking into consideration those hazards that are more easily detected and represent a lower
1986 overall risk. FMECA allows the estimation of a risk priority number (RPN) for the ranking of identified

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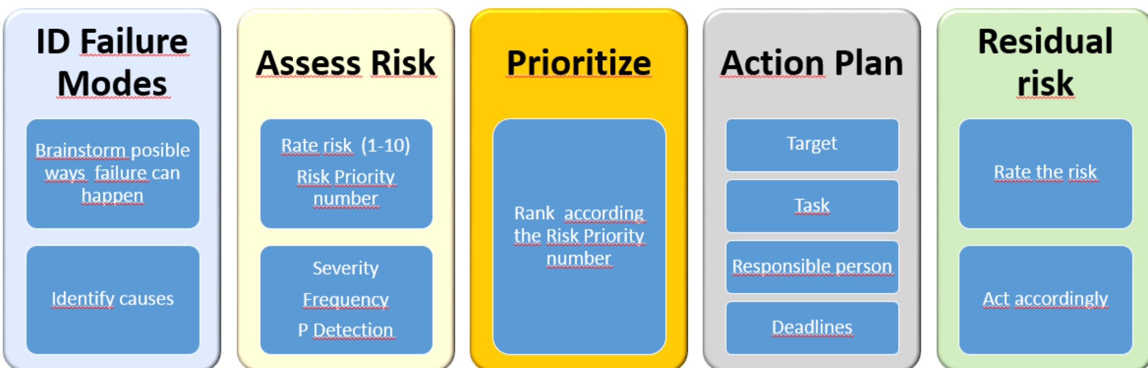
1987 risks [5]. The estimated level of risk should take into account the benefits, possible alternatives and costs
1988 associated with reducing risk further. An organisation should have a clear policy regarding risk
1989 acceptance (see Figure 2.2).
1990 Undertaking risk assessment at various stages helps to define requirements and alternatives, aids
1991 the process of supplier selection and helps to determine the scope and extent of validation. The
1992 methodology has been used for tissue and cell banking for specific process steps [6] and for reviews of
1993 an entire process [7].
1994 Risk assessment is not a once-only process but a cyclical one (Figure 2.3). Risk assessment should
1995 be followed by risk avoidance and reduction (if possible) and continuous re-evaluation of residual risk.
1996 Guidance on quality risk management is provided in Part III Q9 of the Rules governing medicinal
1997 products in the EU, Volume 4: *EU Guidelines for good manufacturing practice for medicinal products*
1998 *for human and veterinary use* [1], in which several well-established risk-assessment methodologies are
1999 listed. Inclusion of this new section in GMP guidance reflects the current thinking that risk management
2000 should be an integral part of quality management.

2001



2002
2003

2004 **Figure 2.2. Failure mode effects and criticality analysis (FMECA)**

2005

Figure 2.3. Cycle of risk assessment

2.18. Root Cause Analysis

Root Cause Analysis (RCA) is a tool used to understand the true cause of why an event has occurred. Through investigation and getting to the root cause, you are able to identify corrective and preventive actions (CAPA) which will prevent any occurrence of the event in the future.

RCA has five purposes:

a. Establish the facts and events that led to the event;
b. Identify what went well;
c. Determine what went wrong;
d. Establish the root cause;
e. Identify CAPA.

When carrying out RCA there are five main factors which need to be considered,

- Plant – what was used, was it calibrated, was it maintained etc?
- People – who was involved, were they trained, competent and capable?
- Procedure – is there an SOP, was it followed, is it correct, has it been validated?
- Premises – location, department, environment etc?
- Product – how many times has this occurred, number of products affected, damage, defects?

When performing RCA, there are additional tools that can be used such as fishbone diagrams, why whys, flowcharts and timelines. Examples of these have been included as appendices 8, 9 and 10.

2.19. Continuity planning

General quality-management responsibilities include budgetary/fiscal oversight and contingency planning to ensure that essential services for patients are not interrupted. Each organisation in the chain – from donation to distribution and biovigilance of tissues and cells – should have a continuity plan in place that details how procurement services, donated tissues and cells and all associated documents will be maintained in the event that activities must temporarily be suspended or permanently ceased. Usually this plan will include a mutual agreement (a service-level agreement or contract) with another organisation for the transfer of tissues or cells, documentation and services in these circumstances.

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2052 **Related documents:**
2053 **Appendix 4. Example of cleanroom qualification**
2054 **Appendix 5. Example of incubator qualification**
2055 **Appendix 6. Example of process validation**
2056 **Appendix 7. Example of method validation (oocyte vitrification) in assisted reproductive technology**
2057 **Appendix 8. Example of 5 Whys root cause analysis**
2058 **Appendix 9. Example of Fishbone root cause analysis**
2059 **Appendix 10. Example of medically assisted reproduction with Fishbone**

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Chapter 3. Recruitment of potential donors, identification and consent

3.1. Introduction

Human tissues and cells may be used in transplantation therapy or for medically assisted reproduction (MAR). They can be obtained from deceased or living donors, as long as the procedure does not compromise the respect due to deceased persons nor endanger the life or health of living donors. Transplantation of tissues and cells can range from life-saving treatments (e.g. serious burns victims, general sepsis due to prosthesis infection, haematological malignancy) to quality-of-life improvements (sight or motion restoration). In addition, donated gametes and embryos may help fulfil a person's wish to have children. Human tissues and cells are also raw material for advanced therapy medicinal products (ATMP).

In order to ensure the safety and success of any transplantation or human application programme, potential unrelated living donors need to be recruited and potential deceased donors need to be identified and referred. In any case, screening must be performed to exclude any contraindications to donation and, in the case of a living donor, to exclude any medical situation that could potentially harm the donor.

Successful donation programmes should at least include [1]:

a. adequate public-awareness strategies, promoting not only organ donation but also tissue and cell donation;
b. effective systems to facilitate the recruitment of living donors in an ethical manner, ensuring their safety and well-being, and the identification and referral of all potential deceased tissue donors to the appropriate organisation (e.g. routine medical chart reviews in every case of in-hospital death);
c. adequate training of professionals involved in the recruitment, or in identification and referral.

Once potential donors are recruited or identified and referred, informed consent is required before donation can take place. Consent is obtained from the donor themselves if alive or from their legal representatives if juvenile or incapable, or for deceased donors either from the donor before death (e.g. donor registries, donor card, advanced directives) or from their relatives; see Appendix 10. The way in which consent is obtained depends on the type of donor, the specific circumstances and the different legal systems for consent. Although the term 'consent' will be used throughout the chapter, the Guide recognises that in some countries, the term 'authorisation' rather than 'consent' is used to enable lawful recovery of tissues and cells.

This chapter describes the process, requirements and key elements at the beginning of the donation pathway for both living and deceased donors.

3.2. Living donors

Some tissues and cells can only be obtained from living donors. This is true for haematopoietic progenitor cells (HPC); for oocytes, spermatozoa, ovarian or testicular tissue and embryos, used in MAR procedures; and for mesenchymal stromal cells and some somatic cells, such as keratinocytes and chondrocytes.

Some tissues can be collected as surgical residues (e.g. placenta, femoral heads removed during surgery to replace a hip joint, heart valves from patients receiving a heart transplant, veins from stripping). In some cases, tissues from a patient must be processed and stored for their own treatment in the future (e.g. skull bone obtained from a decompression craniotomy, parathyroid tissue to be reimplanted in case of insufficiency after its removal during the thyroidectomy, oocytes as part of specific programmes to preserve fertility in oncological female patients).

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2105 Depending on how the tissues or cells will be used, their clinical application can be described as:
2106 autologous: when the tissues or cells procured from a patient are used for the patient's own treatment;
2107 allogeneic: when the tissues or cells donated by one person are used for the treatment of another person.
2108 In this case, the material can be donated for:
2109 i. an intended recipient, who can be related or unrelated to the donor (e.g. HPC);
2110 ii. an undirected recipient, unrelated to the donor (e.g. amnion).

2111 In the case of MAR, depending on the origin of the gametes and embryos, the donation can be
2112 classified as:
2113 iii. partner donation (donation between a couple who declare that they have an intimate physical
2114 relationship);
2115 iv. non-partner donation.

2116 The selection of a living donor must be based on a risk–benefit analysis for both donor and the
2117 recipient. In the case of surgical residues, there is no risk to the donor derived from the donation itself.
2118 In some instances, donation may occur years after the initial selection and registration (e.g. in
2119 HPC donation, when potential donors are included in a registry and the donation only takes place if a
2120 matching recipient needs it).

2121 **3.2.1.Donor recruitment**
2122 Donor recruitment is a continuous process. It begins with increasing public awareness by
2123 educating society, at local and national levels, about the benefits of the clinical applications for different
2124 tissues and cells. Recruitment drives can focus on altruism, solidarity and social engagement, based on
2125 the principles of voluntary unpaid donation.
2126 One way to increase awareness is to explain the benefits for the recipients of donated tissues and
2127 cells. Success stories describing patients' experiences and testimonials of family members may drive
2128 people to consider whether tissue or cells donation is right for them. However, public campaigns should
2129 aim to increase the number of undirected donations and registration on living donor registries.
2130 Publicising and/or advertising the need of donated tissues or cells for a given patient should be
2131 discouraged (in some countries such activities are forbidden/regulated by law).
2132 The most common instances of donation from living donors where donor recruitment plays an
2133 essential role are the donation of HPC and of gametes and embryos for MAR procedures.

2134 *3.2.1.1. Recruitment of haematopoietic progenitor cell donors*
2135 Donation of HPC should be voluntary and unpaid, and informed consent must be obtained in the
2136 preliminary steps. Successful HPC transplantation depends on matching the donor and recipient for
2137 human leukocyte antigens (HLA). If a patient needs an HPC transplant, their siblings and close relatives
2138 should be screened to determine if their HLA types are compatible. If the donor is under the age of 18,
2139 specific consideration is needed (see Chapter 22).
2140 For patients without a matching family donor, there is a possibility that an HLA-compatible
2141 voluntary donor can be found. Therefore, it is of crucial importance that volunteer donors are recruited
2142 and registered on HPC registries around the world, particularly from diverse ethnic communities.
2143 National HPC registries and cord blood banks are linked internationally using tissue-typing to establish
2144 rapid identification of potential donors.
2145 When a person volunteers to be an HPC donor, including expectant mothers, an initial evaluation
2146 is performed. Suitable donors are HLA-typed and their details are placed on a donor registry. Potential
2147 donors are requested to inform the registry in case of any change to their health status that could
2148 contraindicate donation or the possible use of the cells, as in the case of umbilical cord blood (UCB).
2149 They will only be further contacted if they are identified as the best possible match for a patient. In this
2150 case they will be requested to attend a donation centre for a full explanation of the procedures by the
2151 clinical staff and a thorough medical examination and blood tests to detect any medical contraindications
2152 to donation.

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Further information on HPC registries and recruitment of HPC donors can be found in Chapters 22 and 23.

3.2.1.2. Recruitment of donors in medically assisted reproduction

With the development of MAR and changes in lifestyles, a growing demand for gamete and embryo donors has emerged. These developments in most countries also reflect changing social values and government financial support. This growth has not always been accompanied by similarly swift development of laws and regulations in the field. Gradually, countries have set legal provisions and/or guidelines based on their own historical, cultural, religious and social traditions and their political and economic situations. Consequently, there are wide variations in the techniques available and permitted by law in each country, and the types of reproductive cell that can be donated. Whatever the situation, national laws must be respected and donors recruited accordingly.

As mentioned previously, gamete and embryo donation can be classified as partner or non-partner donation depending on the link with the donor. This chapter will focus on the recruitment of non-partner donors.

The practice of gamete and embryo donation is complex, and multiple ethical rules, legal restrictions, medical facts and social and psychological consequences intermingle. Things are further complicated by such factors as:

- the reasons why a person donates, whether for the treatment of others or for a scientific purpose;
- what kind of reproductive cells are donated: donation of oocytes or sperm or embryos commits the donor to a different investment in the medical interventions required and the risk of harm;
- the level of anonymity, whether a known donor, identifiable or anonymous;
- the level of compensation schemes for donors;
- how often a donor can donate and how many offspring may derive from a single donor.

The scarcity of donor gametes has stimulated discussion about acceptable systems of recruitment, especially since it has become evident that different clinics and countries are using different approaches to recruit donors, with various modes of compensation for donors (e.g. compensation of proven expenses, compensation through lump sums, oocyte-sharing schemes). However, it is essential to emphasise that the donation of reproductive material should strictly follow the same principles of being voluntary and unpaid as for other donations of tissues or cells. Any type of reward, benefit or incentive may be a threat to voluntariness and compromise the degree of confidence that can be placed on medical interview (see Chapter 27).

The donor-recruitment activity, whether performed by a public health system or by private clinics (where allowed by national legislation), must be authorised by the Health Authority.

As with any living donors, it is essential that gamete donors are entered into registries, not as a database of potential donors, such as for HPC, but to guarantee traceability and transparency of practice (see Chapter 15). In addition, registries allow adequate follow-up of the donors, including the collection of data on serious adverse events or adverse reactions in donors, recipients and in the resulting offspring (see Chapter 16).

Further information on recruitment of gamete and embryo donors can be found in Chapter 27.

3.2.2. Consent for living donation

The donation process differs depending on whether a person donates tissues and cells for the benefit of others, and accepts risks and inconvenience that they would not otherwise face, or donates while undergoing a medical intervention for their own benefit. Donation implies an altruistic act and, to some extent, a trade-off between individual well-being and societal utility. As a consequence, there is potential for the abuse and exploitation of individual donors. Obtaining individual consent, in any situation of donation, either collection of surgical residues or tissues and cells procurement, is crucial to assure that donation conforms to professional ethical standards and the individual's own goals and values.

2201 Donation of tissues or cells must only be carried out after the person concerned has given free,
2202 informed and specific consent, either in written form or orally before an official body.
2203 Consent should be recorded and/or documented in the donor/patient's record. Informed consent
2204 must be discussed with the donor or their legal representative in a language and with terms they can
2205 understand. The record should mention that the prospective donor has understood – and, where
2206 appropriate, their legal representatives or their relatives have understood – the information given, had
2207 the opportunity to ask questions, received satisfactory answers and confirmed their position on donation.
2208 Some examples of forms to obtain consent for MAR may be found in Appendices 11, 12 and 13.
2209 Recruitment of persons not able to consent should never be done through public registries. In
2210 addition, in some countries, specific regulations restrict donation in these circumstances (e.g. some
2211 countries do not allow procurement of peripheral blood progenitor cells from minors and/or
2212 administration of growth factors).
2213 In accordance with Article 14 of the Additional Protocol to the Oviedo Convention, no tissue or
2214 cell procurement may be carried out on a person who does not have the capacity to consent.
2215 Exceptionally, and under the protective conditions prescribed by law, the removal of HPC for allogeneic
2216 transplantation from a person who does not have the capacity to consent may be authorised provided the
2217 following conditions are met:
2218 a. there is no compatible donor available who has the capacity to consent;
2219 b. the recipient is a brother or sister of the donor;
2220 c. the donation has the potential to be life-saving for the recipient;
2221 d. the authorisation of their representative or an authority or a person or body provided for by law
2222 has been given specifically and in writing and with the approval of the competent body;
2223 e. the potential donor concerned does not object.

2224 More recently, in the context of haplo-identical HPC transplantation, these conditions have been
2225 extended to include other *first- or second-degree relatives* (not only brother or sister) when no other
2226 donor is available and all the other conditions are fulfilled.
2227 Potential donors must beforehand be given appropriate information as to the type(s) of tissues or
2228 cells to be procured, the purpose and nature of the intervention, its consequences, possible side-effects
2229 and risks, whether the tissues or cells will be processed and stored, and the purpose or use to which the
2230 tissues or cells will be put. All relevant information should be given because consent must be specific
2231 and cannot be assumed for uses of tissues or cells about which the donor has not been informed. Donors
2232 must be given all the information needed to ensure that they understand all potential purposes and
2233 benefits for the recipient before they give consent. Full understanding of the consent is particularly
2234 important when practices may be controversial (e.g. the use of gametes or embryos for research
2235 purposes). Similarly, some donors may not wish to donate tissues or cells to a commercial organisation
2236 where, for example, their donated tissues or cells may be used as starting material for developing
2237 therapies in a commercial setting.
2238 Potential donors should have an advocate, who must not be involved in the treatment of the
2239 recipient. Information about potential risks for the donor and risks and benefit for the recipient must be
2240 clearly understood before consent is given. Where relevant, they must also be informed that the results
2241 of the qualification of tissues and cells for transplantation and the transplant itself are uncertain because
2242 they depend on many factors, including the recipient's situation. The donor should also be provided with
2243 information about psychological support in case the transplant does not lead to survival or cure of the
2244 recipient.
2245 The scope and duration of the consent must be stated explicitly. When the tissues and cells are to
2246 be stored, the consent should include information about the storage time and the policy once the period
2247 has expired. In the specific case of gametes and embryos, some countries have regulations on the
2248 duration of storage.

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2249 The interview for consent should be conducted in a suitable environment. The interviewer should
2250 have received specific training for this purpose to be able to consider the donor's needs and to answer
2251 questions about donation and transplantation processes.
2252 Potential donors, or their legal representatives, or their relatives, should be informed that tests
2253 will be performed to evaluate the possible existence of any transmissible diseases that would be a risk
2254 to the recipient, and the action to be taken in the case of a positive result. They should be then informed
2255 and receive adequate counselling. All results of the medical evaluation should be kept available to the
2256 donor. If a potential donor is found to be unsuitable, the reasons and the results of the medical assessment
2257 should be discussed with them and advice should be given on recommended actions regarding their
2258 health. If the findings do not exclude the potential donor but may have implications for the recipient
2259 (e.g. an HPC potential donor carrier of a haemoglobinopathy), the transplant centre must also be
2260 informed.
2261 In the case of HPC donors, consent must be obtained at several stages: before HLA typing, before
2262 donor testing and before the conditioning regimen on the recipient has begun. The interview must
2263 include information about donation procedures, their risks and side-effects, the procurement method,
2264 and the need to administer growth factors when peripheral HPC is being procured. In the case of cord
2265 blood donation, consent is usually obtained months before the delivery and should at least be obtained
2266 before the mother goes into labour to avoid interfering during the delivery.
2267 Documentation must be made available to the cell or tissue establishments that receive the
2268 procured material for processing and storage to confirm that detailed consent has been duly given (see
2269 Chapter 2).
2270 Donors must be informed that they may withdraw consent at any time. In case of HPC donors,
2271 they should be informed of the possible consequences for the recipient if they withdraw their consent
2272 once the conditioning regimen on the recipient has already begun. Ultimately, this situation cannot be
2273 used to coerce the donor and their final decision must be respected.
2274 In the case of autologous donation, the patient must be informed about options and the balance of
2275 risks and benefits of the procedure. The consent must specify what would happen if the clinical
2276 application could not be performed for any reason (e.g. contamination of the transplant or if the patient's
2277 condition contraindicated application). In such instances, the consent should indicate whether the tissues
2278 or cells could be used for the treatment of others, for research or must be disposed of.
2279 Consent should be obtained from the patient before procurement; but, in some circumstances, this
2280 may not be possible (e.g. emergency craniotomies) and the decision to procure and, if necessary, to
2281 process the tissues or cells should be taken by the medical team.
2282 In the case of planned surgical procedures where it may be possible to donate residues, consent
2283 should be obtained before procurement. As is the case for all tissue or cell donors, the donor should be
2284 informed beforehand about tests to determine the suitability of the transplant, and consent should include
2285 information about the processing, storage and intended use of the donated material. The consent must
2286 also specify the fates of tissues or cells if their clinical application cannot be performed for any reason.
2287 Donors, or their legal representatives, or their relatives, should be aware of the importance to
2288 recipient safety of providing the medical staff with information about any medical condition of the donor
2289 that may occur after donation.
2290 They should be informed about, and give consent for, the use of personal data in computerised
2291 data processing.

2292 3.3. Deceased donors

2293 Tissues donated by a single donor can be used to treat as many as 100 recipients. Tissues donated by
2294 deceased donors typically include the following tissue types:
2295 a. musculoskeletal: bones, tendons, ligaments of the upper and lower extremities, menisci, fascia,
2296 cartilage;
2297 b. cardiovascular: heart valves, blood vessels, pericardium;
2298 c. skin: split thickness (typically, only the epidermis) and full thickness (epidermis and dermis);

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d. ocular: corneas, sclera;
e. specific cell types from certain tissues (e.g. limbal stem cells) or from certain organs (pancreatic islets, hepatocytes).

In order to guarantee the success of any donation programme from deceased tissue donors it is essential that hospitals (and other centres where potential tissue donors may be found) have a system to identify potential donors, to check the presence of donor's consent or lack of donor's objection expressed by donor before their death or to obtain consent from family members or relatives of potential donor and refer donors to the appropriate procurement agencies or tissue establishments.

Communication with bereaved family members or relatives will require clear and sensitive procedures or protocols with consent obtained by appropriately trained specialists in donation (see Appendix 8).

3.3.1. Identification and referral of deceased tissue donors

Tissue donation depends on commitment and the development of strong working relationships between tissue establishments, procurement organisations and hospitals and other centres where potential tissue donors may be found, such as medical examiners' offices, mortuaries, coroners' offices, forensic institutes, funeral homes, emergency medical services, and nursing or retirement homes.

It is recommended that any potential donors considered for organ donation are also referred for potential tissue donation. Furthermore, it is recommended that all deaths (typically hospital but also community deaths) should be routinely referred to a donor co-ordinator, procurement organisation or tissue establishment, regardless of the age of the patient, the cause of death or the known wishes of the donor or their family to become a tissue donor. Routine referral of all potential donors with no known medical contraindication gives every individual the opportunity to donate and allows for the standardisation of donor-selection criteria.

In every deceased donation programme, it is essential to identify all of the parties who may be involved, in order to co-ordinate and facilitate the process and maximise the opportunities for successful procurement. The key parties are summarised in Table 3.1.

Before procurement, checking the donor's identity is an essential prerequisite.

The medical suitability of potential donors should be investigated using medical records, interviews with medical staff who treated the donor (attending physician, general practitioner, nurse) and relevant information provided by the donor's relatives or legal representatives, family doctor or other persons who have information about the donor's behavioural and medical history. Selection criteria must be taken into consideration before a donor is accepted, and risk factors such as sexual behaviour, travel and exposure to sources of infection must be evaluated. These criteria may vary depending on the type of tissue to be procured for human application (see Chapter 4 for general criteria and Part B for tissue-specific criteria).

In the event that a health facility does not have the means to manage a potential tissue donor or is not licensed/authorised for tissue procurement by their Health Authority, arrangements should be made, where possible, for transfer of the potential donor to a suitable hospital or procurement centre.

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2339 **Table 3.1. Key parties in tissue-donation programmes and the challenges and opportunities they offer**

2340

	Opportunities	*Challenges*
Public	• learn about donation and the benefit of transplantation to recipients • promote solidarity and altruism • express wishes to family and friends in relation to donation	• lack of knowledge about donation • unwillingness to face death • fear that an expressed desire to become a donor may interfere with medical care • lack of trust in the fairness of the donation and transplantation system
Donor family	• honour the wishes of the loved one • make some sense out of the death of a loved one • find comfort in knowing that donated tissues of the loved one helped save or improve lives • gain some control after the death of a loved one • know that a part of the family member 'lives on' • recognition and gratitude from society and recipients	• grief • lack of understanding or knowledge about donation • fear of disfigurement • not knowing the wishes of the loved one • lack of trust in the medical profession or fairness of the donation system
Hospital staff	• support wishes of the donor and their family • increase availability of tissues for patients in need • contribute to public awareness of donation and transplantation	• discomfort or lack of knowledge about donation • lack of trust in the donation and transplantation system • not acknowledging the value of tissues • increased workload • lack of acknowledgement from those involved in procurement • lack of resources
Transplant co-ordinator	• support wishes of donor and their family • maximise donation opportunities – tissue donation may benefit as many as 100 patients per donor • increase availability of tissue for patients in need • contribute to public and professional awareness of donation and transplantation	• concern that tissue consent will have an impact on consent to organ donation • lack of knowledge about tissue donation and its benefits • increased workload
Coroner	• mutually beneficial for coroner and procurement team/tissue establishment, who may share findings, tissue/blood samples and test results to help investigations • support wishes of the donor and their family • increase availability of tissues for patients in need	• lack of knowledge about donation • concern over potential impact on death investigation
Funeral home director	• extend support to bereaved families • raise awareness by including donation information in funeral homes and obituaries • allow procurement of certain tissues to occur in funeral homes • contribute to the conditioning of the body • increase availability of tissues for patients in need	• lack of knowledge about donation • fear of difficulty in preparing body for funeral services • increased time and costs • delay in funeral services
Retirement and nursing homes	• support wishes of the donor and their family • raise awareness by including donation information • allow procurement of certain tissues to occur on their premises • increase availability of tissues for patients in need	• lack of knowledge about donation • lack of trust in the medical profession and in the donation and transplantation system • many of the residents may be affected with conditions that contraindicate donation

2341 **3.3.2. Consent for deceased donation**

2342 Before procurement of tissues from a deceased person can take place, consent to donation must
2343 be obtained and recorded in order to ensure that their wishes are fulfilled. In some cases, the deceased
2344 person may have expressed their wishes while alive through a donor registry, donor card or advanced
2345 directive. In other cases, it is members of the family of the deceased person who decide if donation was
2346 in accordance with the person's wishes, values and beliefs, and whether the deceased had expressed an
2347 objection to donation during their lifetime and give consent accordingly.

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It is important to emphasise that consent must be specific. Therefore, donors' relatives or legal representatives must be given all the information needed to ensure that they understand all potential uses, including processing and storage, before they give consent. The specificity of consent is particularly important when the donated tissues and cells may not be used for transplantation, for example, when the donated tissues may be sent to a biobank for research or used as starting material for advanced therapies (ATMP) by commercial organisations (see Chapter 1).

3.3.2.1. Legal consent systems

Consent for the donation of organs and tissues from deceased donors is subject to national legislation and regulation in each country.

There are two legal consent systems for expressing consent to donation. Opting-in is a system in which consent to donation has to be obtained explicitly from the donor during their lifetime or from an authorised individual (usually the next of kin). Opting-out is a system in which consent to donation is presumed, no objection to donation has been registered by an individual during their lifetime or is known to have existed, or consent is inferred by the donor's family who could testify the donor position. In practice, variations exist within both systems, and the relatives may play a prominent role in the decision.

An example of an opt-in system that allows the donors themselves, or their relatives after their death, to give consent is the Human Tissue Act 2004, which applies to England and Northern Ireland. According to this legislation, consent for removal of tissue from deceased donors must be given by the person when they were alive or, after their death, by their legally authorised representative or a "person in a qualifying relationship to the deceased". The Act prescribes a hierarchy of qualifying relationships (ranked from highest to lowest: spouse or partner, parent or child, brother or sister, grandparent or grandchild, niece or nephew, stepfather or stepmother, half-brother or half-sister, long-standing friend) and states that consent should be obtained, where possible, from the person ranked highest in the hierarchy.

The opt-out system was introduced to help meet the shortfall in organs and tissues available for transplantation. However, in most programmes, if the family is against the idea of the donation proceeding, tissue procurement will not proceed; this is called a 'soft' opt-out system.

Even if the legislation is based on a presumed consent or opt-out system, the family and/or other persons who knew the donor well must be engaged in the donation process to provide information on medical and behavioural risks about the potential donor to ensure the safety of donated material.

Table 3.2 gives an overview of national consent systems in Europe. The information is reproduced from a survey conducted by the European Commission and was up to date in August 2014 (Directive 2010/53/EU Implementation Survey). Of the 36 answering countries, it appears that the majority (21 countries) have an 'opt-out' system, 12 countries have an 'opt-in' system. Three countries have a mixed system either combining elements of both 'opt-in' and 'opt-out' or, as in the United Kingdom, with an 'opt-in' system in three of the four UK administrations (England, Scotland and Northern Ireland), and 'an opt-out' system introduced in Wales from December 2015.

Different countries have different procedures to help people express their wishes regarding organ and tissue donation [2]. In each country, national legislation (or, if this is lacking, operational policies) should make clear what evidence (i.e. written or oral) is valid in their country to confirm consent or objection to organ and tissue donation.

Among the means to express wishes regarding organ and tissue donation after death are donor cards and donor registries. Individuals who have donor cards are also often simultaneously recorded in the national donor registry. Consent to donation recorded on a donor card or in a registry may contain detailed information, e.g. consent or not to various types of donation (donation of specific organs or tissues). In some countries, those who apply for a passport or driving licence have to state whether or not they are willing to donate organs, tissues and cells after death. Advance directives ('living wills') may also enable individuals to state prospectively under which medical conditions they do not want to receive life-sustaining therapy and allow documentation of people's wishes related to donating organs and tissues after death.

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All national systems should enable individuals to modify or withdraw their consent or objection at any time. This ensures that the most recent information about an individual's wishes is recorded in some way and is available at all times should an enquiry be received from a doctor or a donor co-ordinator involved in the donation process.

3.3.2.2. Establishing consent in other circumstances

In countries with no legal framework for consent to donation, or where a potential donor is not able to express their donation preference, for example a minor, the decision is, as a rule, left to the family of the potential donor, based on the assumption that the family would respect and represent the potential donor's wishes. Alternatively, consent may pass to those who are the nominated legal representatives of the potential donor, according to the laws of the country.

In some circumstances (e.g. when death occurs in suspicious circumstances or as a result of an illicit act), authorisation to proceed must be given by a coroner, a judge or a family court to avoid the procurement interfering with an investigation, even if the family has consented to donation.

In other circumstances, when a person was to become a donor (expressed wish or absence of refusal/objection), if no relatives can be found or contacted, national procedures and regulations should enable organ and tissue procurement where possible, but only if sufficient medical, social and behavioural information to support safe donation and transplantation can be obtained by other means (e.g. from family doctor or friends). If this level of information cannot be reached, donation should not be considered, as it does not guarantee the safety of the recipient.

Table 3.2. Legal provisions in European countries for consent to/authorisation of organ donation from deceased persons

Country	***National consent system***	***Donor registry***	***Non-donor registry***
Austria	opting-out		×
Belgium	opting-out		×
Bosnia Herzegovina	opting-out		
Bulgaria	opting-out		×
Croatia	opting-out		×
Cyprus	opting-in	×	
Czech Republic	opting-out		×
Denmark	opting-in	×	×
Estonia	opting-out	×	×
Finland	opting-out	n/a	n/a
France	opting-out		×
Germany	opting-in		
Greece	opting-out		×
Hungary	opting-out		×
Iceland	opting-in	n/a	n/a
Ireland	opting-in	n/a	n/a
Italy	opting-out	×	×
Latvia	opting-out	×	×
Lithuania	opting-in	×	
Luxembourg	opting-out	n/a	n/a
Malta	opting-out	×	

Montenegro	opting-in		
The Netherlands	opting-in	×	×
Norway	opting-out	n/a	n/a
Poland	opting-out		×
Portugal	opting-out		×
Romania	opting-in	×	
San Marino	opting-out	n/a	n/a
Serbia	opting-in	×	
Slovakia	opting-out		×
Slovenia	mixed system	×	×
Spain	opting-out	×	×
Sweden	mixed system	×	×
The Former Yugoslav Republic of Macedonia	opting-in	n/a	n/a
Turkey	opting-in	×	
United Kingdom	mixed system (opting-out in Wales)	×	×
×: system in use	***n/a: data not available***		

3.3.3. Approaching the families of potential tissue donors

Grief and mourning are crucial processes to help cope with the death of a loved one. When approaching a bereaved family to seek consent or information on the position of the deceased regarding donation, the many aspects of acute reactions to grief following a death should be understood in order to deal with the circumstances sensitively and in an understanding way. Donation professionals should learn to navigate the environment of acute grief to obtain a decision about donation that is best for the family. By applying their skills and experience, donation professionals can support the family in their mourning and provide appropriate help. The physician or donor co-ordinator who is in charge of approaching the family for donation should have accurate knowledge of the purpose and needs in tissue transplants.

3.3.3.1. Conversations with the family

Conversations with a family about organ donation do not generally differ from conversations related to tissue donation. Therefore, it is best to perform interviews about the donation of organs and tissues in a single interview session with the family, allowing them time to reach a decision with which they are comfortable.

It is frequently impractical to discuss donation with a large number of family members, and it is recommended that participating family members should be limited to the lawful next of kin and/or those who are key to making the decision, taking into account the legal framework in place and cultural or religious practices. This should be explained to the other family members so that they do not feel excluded.

When there are social, cultural or language barriers or difficulties, the support (if there are no other possible helpers) of interpreters or friends of the potential donor who have a greater level of integration or similar religious sympathies may be beneficial for the family. These persons should be previously informed about the donation, so that they can support the family and champion a favourable attitude towards donation; they should not be limited to making a simple translation.

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2446 The conversation should be planned, carried out at the right time, in the right place and by trained
2447 people. Proper preparation for the conversation reduces the likelihood of errors and the need for
2448 improvisation [3, 4, 5, 6]. The discussion should take place in an environment that helps facilitate the
2449 conversation, perhaps located close to the place where their loved one died, to give family members the
2450 opportunity to say goodbye. It is important to provide the family with a quiet room, where they can
2451 speak freely. It is also advisable to have resources that meet the minimum needs (e.g. telephone,
2452 handkerchiefs, water, food).
2453 In certain circumstances, relatives of potential tissue donors may be interviewed over the
2454 telephone. Before the interview, the co-ordination staff should be very careful and verify whether the
2455 interlocutor has been informed of the death of their relative. It may happen that the hospital staff did not
2456 reach them before. Such interviews need to ensure that the conversation takes place when the relative is
2457 in a private space and preferably not in an unfamiliar environment, such as a hospital. Telephone
2458 conversations can make it more difficult to offer reassurance and support to a family since there is little
2459 opportunity to demonstrate a personal touch, which can increase the emotional distance. However,
2460 trained personnel should be able to find appropriate words, and respect silent moments, to provide
2461 support under these circumstances.
2462 The doctor or donor co-ordinator who is conducting the conversation with the relatives should
2463 respect their grief. This type of conversation requires interpersonal skills, sensitivity and empathy. In
2464 situations when there is a huge pressure on medical staff, conversation with families can become
2465 difficult, rushed or insensitive.

2466 *3.3.3.2. Family objections*

2467 Conversations about tissue donation aim to fulfil the will of the deceased donor and to obtain
2468 family consent or support for donation. Regardless of the legal position, conversations must aim to
2469 achieve a decision that is acceptable and accepted by the relatives. Agreement to donation must not be
2470 coerced or conditional, nor should it be achieved under pressure or by offering any financial inducements
2471 or other material benefit.
2472 It is difficult to proceed with donation when a family is strongly against it, even if there is evidence
2473 that their deceased family member wished to be a donor. The family has the right to express their opinion
2474 about donation, and clinicians need to make a balanced decision to continue with the procurement
2475 without the support of the family and risk damaging the emotional health of the relatives, incurring
2476 possible bad publicity and a loss of public confidence in the donation programme, or accept that it is not
2477 feasible to follow the wishes of the deceased and abandon the donation process.
2478 It might be helpful to use the following when discussing refusal with the family:

2479 • If the family claims that the deceased patient did not agree to donation or had changed their
2480 mind, explore the basis on which the family gives such a statement.
2481 • When the family does not know anything about the attitude of the deceased to donation, discuss
2482 whether the deceased helped people generally, e.g. as a blood donor or giver to charity, and how
2483 donation could help many people to benefit from a transplant.
2484 • The experience of interviews with families suggests that some difficulties and possible
2485 opposition may occur in procurement of tissues from ‘visible places’ like skin, bone and, in
2486 particular, eyes when family members fear disfigurement of the body. In these situations,
2487 reassure them that the deceased’s body will be fully respected. If necessary, some technical
2488 aspects of procurement should be explained, for example the use of specific surgical incisions
2489 and sutures or suitable prostheses or artificial eyes or bones. Reassurance should be given that
2490 they should not notice anything if they see the deceased person after procurement, albeit there
2491 can be rare problems such as bruising or bleeding, and they should be made aware of such
2492 possibilities.
2493 • In the case of religious concerns, offer a consultation with a religious leader or representative.
2494 • Give special attention to cases of dissatisfaction with the healthcare provided, record the
2495 complaints but explain that the issue of donation should be kept separate.

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- Identify the persons involved in the refusal to donate and their role within the family, and attempt to communicate with them separately to understand and try to address their concerns.
- Identify whether a disagreement to donation by individual family members is based on conflicts between family members which come to light when a person has died. In this case, try to separate the conflict from the issue of tissue donation.

3.3.3.3. Informing the family

There is often debate about the amount of information a donor family should receive, how much information is enough and when does it become too much. One opinion is that the family should be given only the information they request; another is that the family must be told absolutely everything: which tissues will be donated, a description of the procurement process including reconstruction, potential uses of the tissue in both clinical practice and research, the method of discard, the potential need for follow-up if some test results are found positive, etc. The first approach has the limitation of not taking into consideration the fact that the family may not know much about donation and will therefore not know what to ask. The latter could cause harm to the family and could risk converting an interest in donation into a refusal. The solution to this dilemma should be found by the donation professionals on a case-by-case basis.

All questions posed by the potential donor family should be answered and, beyond that, professionals should apply their own judgement to decide how much information the family would want or need to obtain. While providing the information, they may observe agitation, frustration or irritability in a family member; this may signal unwanted stressful information and suggest reconsideration of how much information is needed or wanted. Ultimately, the amount of information made available to donor families in order to obtain consent should vary with the type of consent being provided and should be increased if the potential application of the donated material is controversial. The interview in any case should be prepared in advance with systematic content, built with the interviewer's owns words. Global and generic sentences may be helpful to raise the main subjects, which the family can investigate further with questions if needed.

It is helpful to ensure that, following donation, the family receives the appropriate care they need. In many countries hospitals have dedicated bereavement teams to provide psychological support, access to social services, administrative support or religious counselling. The clinical team should establish whether there are any specific religious or spiritual requirements of the family and whether the family wishes to retain keepsakes such as locks of hair or handprints. Finally, establish whether the family wishes to assist with the final preparation of the body following donation, such as washing or dressing in certain items of clothing.

3.4. Conclusions

The continuing development of transplantation medicine gives hope to many patients in need. However, the need for tissues and cells for clinical application cannot be met only by autografts or surgically discarded tissues. The ability of a tissue establishment to meet patients' needs requires multiple efforts to increase tissue and cells donation activity. These efforts involve organisational measures, the development of proactive donor recruitment and identification programmes, and the engagement of many parties, including the general public, hospital staff, coroners, procurement organisations and tissue establishments. By establishing strong links with and co-ordination between all these parties, and by adequately training personnel to acquire the necessary medical expertise and key social and emotional skills, tissue establishments can ensure the success of the tissue and cell donation programmes.

Since tissues and cells come from a human being, either living or deceased, it is necessary to ensure that donors have the autonomy to decide freely about matters that are essentially their own choice. Obtaining informed consent is relatively straightforward when donors are alive. It is essential, however, that they fully understand the risks and consequences of the donation procedure and the final use that will be given to their donated material. In the case of deceased donors, it is less clear how respect for autonomy applies but, ultimately, it is crucial that the wishes and best interest of the potential donor are

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scrupulously respected. This chapter has aimed to offer practical guidance for obtaining consent in all possible contexts.

3.5. References

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Related documents:

Appendix 10. Sample consent form;
Appendix 11. Sample consent form (women);
Appendix 12. Sample consent form (women);
Appendix 13. Sample consent form (men).

Chapter 4. Donor evaluation

4.1. Introduction

Since the development of tissue and cell transplantation as clinical treatments the key objectives of all parties involved in the process have been to ensure product safety and quality as well as donor safety [1, 2]. The rationale for donor screening is twofold: firstly, to minimise the risk of transmitting disease to a recipient and, secondly, to exclude any tissues or cells whose quality may be adversely affected by a characteristic, including medical or other conditions, of the donor. When dealing with living donors, an important part of the evaluation should be to assess whether the procurement process itself could be harmful to the donor. It should also be considered whether it is necessary to have arrangements in place for long-term follow-up of living donors after procurement. Special consideration is required for paediatric donors. These aspects of living donation are detailed in Chapter 3 on recruitment of living donors, Chapter 22 on haematopoietic progenitor cells (HPC), Chapter 27 on medically assisted reproduction (MAR) and Chapter 28 on fertility preservation.

There are two main donor types, with different risks and benefits resulting from the donation: autologous and allogeneic. Allogeneic donors may be living or deceased. The evaluation of autologous donors is a special situation as the donor is the person being treated for a disease and the acceptance criteria should take this into consideration in decision making.

4.1.1. Donor evaluation

The two main objectives of donor evaluation are:

a. To obtain information about the donor to identify absolute and relative contraindications to human application that may pose risks for a recipient;

b. To ensure that the donation will not cause harm to a healthy living donor.

To meet objectives a and b above, the following information should be evaluated:

i. medical history (including genetic disease and a family history of disease);
ii. social history (personal and behavioural information, including travel history);
iii. physical examination;
iv. psychological examination (living HPC and non-partner MAR donors) – see Chapters 22 and 27;
v. tests for markers of transmissible disease, as detailed in Chapters 5 and 10;

During donor evaluation, confirmation of the validity of the consent and of the donor identity are essential steps (see Chapter 3). For European Union (EU) member states, the selection criteria for deceased donors (including additional exclusion criteria for deceased child donors) and living donors of tissues and cells are specified in Annex I/III of Directive 2006/17/EC. These criteria are the minimum, and individual member states can set additional criteria as necessary.

4.2. Assessment of potential autologous donors

4.2.1. General evaluation

The evaluation of autologous donors is based on the disease/condition being treated. The donor eligibility criteria for autologous donors may be very different from the criteria for allogeneic donors [1] because the direct benefit of transplant for their medical condition may outweigh potential risks associated with donating cells and tissues for autologous use. The clinician caring for the donor is making the decision on autologous donation/application according to guidelines and relevant scientific

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data. As the autologous donor is being treated for the disease/condition in question, the relevant medical history, results of laboratory tests and physical examination results are all available to the clinician. Eligibility for donation is evaluated on an individual basis, taking into consideration the possible risks and benefits.

4.2.2. General contraindications

There are no absolute contraindications for autologous donors. The potential benefits and risks should be analysed on an individual basis and a decision made by the clinician in charge (e.g. auto-transplantation of ovarian tissue carries the risk of cancer cells being present in the tissue). This should be clearly documented and communicated to the patient.

If the procured tissues or cells will be processed and/or stored, screening for the same biological testing for mandatory markers must apply as for an allogeneic living donor (see Chapter 5), although the results are not necessarily a contraindication for autologous donation. Potential or proven infectious (i.e. HIV/HBV/HCV-positive) materials collected from autologous donors should be handled in such a way that the risk of cross-contamination with tissues and cells from other donors within the tissue establishment is minimised. The risk of transmission of infection to personnel, during procurement, processing and storage of these cells, should also be considered. Written standard operating procedures (SOPs) should be present for these situations (see Chapters 7, 8 and 9).

4.3. Assessment of potential allogeneic donors

4.3.1. General evaluation

Allogeneic donors can be living donors related to the intended recipient, unrelated voluntary living donors or deceased tissue donors. The most common tissue donated by living donors is the femoral head, surgically removed during a hip-replacement procedure. Also recognised as living donation are the *post partum* collection of the amniotic membrane donated by mothers at the time of delivery and heart-valve donation from the discarded heart of a heart transplant recipient; such a person can also be called a 'domino donor'. Minimising the risks to donors of highly matched HPC products for related recipients can be challenging and, in general, are much more difficult than minimising the risk of subjects donating matched or unmatched cells or tissues for strangers. The World Marrow Donor Association and similar organisations are active in many countries to help protect the health and safety of unrelated HPC donors [1,2].

The medical and social history of a potential donor, either living or deceased, must be investigated for factors that increase risks of infection with transmissible diseases and any other conditions that may affect tissue quality and safety.

4.3.1.1. Sources of information

The information on medical and social history required for donor evaluation should be obtained directly from living donors. Additional information (where applicable) should be sought to ensure safety for the recipient, and this should be the same as the information obtained for deceased donors, as given below. The types and extent of records to be obtained should vary depending on the type of donor.

In addition, the health risks for the living donor must also be considered by a clinician not involved in the treatment of the potential recipient, to avoid conflicts of interest (except in the case of surgical residues).

Procurement of tissues from deceased donors takes place after circulatory arrest. Thus, the time available for full donor evaluation is limited. Several sources of information should be used to gather medical and social history about deceased donors (see Table 4.1).

2654 **Table 4.1. Sources of information and types of record for donor evaluation**

Sources of information	*Types of record*
i. interview with family and friends/close acquaintances ii. interview with attending clinician and nurse, as well as the healthcare provider iii. detailed review of the medical notes (see types of record, to the right) iv. general practitioner notes v. physical examination findings vi. autopsy findings (for deceased donors), which must be communicated as soon as possible after procurement vii. tests for infectious markers (see Chapters 5 and 10) and other relevant test results (see types of record, to the right)	i. emergency room and emergency medical transport (ambulance) records ii. admission records, progress notes, clinician's orders/notes and nursing observations iii. surgical records iv. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling) v. discharge summary or death certificate (for deceased donors to confirm cause of death or to determine whether an autopsy is planned) vi. results of laboratory tests (microbiology, chemistry, haematology, virology, toxicology, genetic screening, pathology) vii. physical evaluation form viii. information relating to transfusions and infusions (to be used for evaluation of haemodilution) ix. radiography/magnetic resonance imaging/computed tomography
For deceased donors whose death occurred outside a healthcare facility, the records listed to the right may also be available and, if so, they should be reviewed	x. police records xi. records from the medical examiner or coroner xii. records from the extended-care facility

2655

2656 Information obtained during this evaluation (see Table 4.1) must be included in the donor's
2657 medical evaluation record. An interview with relatives of deceased donors should be undertaken, bearing
2658 in mind that, under emotional stress, some details might be forgotten. Even when donor relatives trust
2659 the interviewer, they may neglect or not disclose this information or may not know the entire truth.
2660 Contact with the general practitioner of the donor and reviewing, where available, hospital records
2661 for historic data or other sources of information (e.g. tumour registry/pathology reports if available) are
2662 important in supplementing and/or confirming information provided by the family. The donor medical
2663 evaluation record should be documented with details of hospital admission (if the donor died in a health
2664 facility); cause of death; medical and behavioural history, including general data such as age, gender,
2665 body weight (if necessary, e.g. to calculate haemodilution), date and time of death; and signs of obvious
2666 medical interventions, i.e. scars, skin or mucosal lesions.
2667 Standardised questionnaires should be used for interviews to ensure that all the relevant
2668 information is obtained (see Appendix 14). The interviews should be performed, documented and signed
2669 by a suitably trained and competent authorised person to comply with national regulations. They should
2670 be held in private and carried out ideally before donation (see Chapter 3). The donation record, whether
2671 paper or electronic (see Chapter 2), must fully and accurately reflect the relevant information gained
2672 from reviewing these records and from discussions with medical or other personnel. Transferring
2673 information from records to a new document carries the risk of transcription or interpretation errors.
2674 These steps must be carried out by well-trained, competent staff.
2675 Careful review of all the collected donor information will help ensure an accurate donor
2676 evaluation and assessment of the risks, including the identification of any potential contraindications for
2677 donation, either absolute or tissue-specific (see Part B for specific chapters). This analysis should
2678 preferably be performed before procurement; but, if this is not possible, the procured tissues and cells
2679 should be quarantined until a final decision is made by the Responsible Person (RP) of the tissue
2680 establishment. In addition, it is the responsibility of the person/team performing the procurement to

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document any suspicious anatomical findings observed during the procurement procedure and to obtain samples for histological examination if relevant.

The HPC donor should be pre-screened for factors that would place them at increased risk due to donation. Pre-screening might include health history questions, physical exam, blood tests and other medical evaluation. If large volumes of blood are to be collected, the donor's haemoglobin should be measured before the donation, and potential donors with low levels should be excluded from donating. If marrow is to be aspirated to manufacture bone-marrow stromal cells or a skin biopsy obtained to manufacture induced pluripotent stem cells, the donor's platelet count and coagulation measures should meet pre-defined criteria to be sure that they are not at increased risk from bleeding [1, 3]. More detailed information about HPC, including paediatric donors and non-partner MAR donors, is given in Chapters 22 and 27.

4.3.1.2. *Donor medical and social history*

4.3.1.2.1. Medical history

a. Current clinical information, e.g. diseases/diagnoses, transfusions/infusions, medication/vaccinations, and cause of death (COD) for deceased donors, should be reviewed. Haemodilution should be assessed in donors with trauma, intra-operative blood loss or ruptured aneurysms, bleeding from oesophageal varices, spleen rupture etc. If haemodilution is $>50\,\%$, serology testing on blood samples drawn at the time of procurement may not be reliable (see Chapter 5 and Appendix 18). When haemodilution is suspected/confirmed, blood samples taken before haemodilution should be used for virology and serology testing. If pre-transfusion/infusion samples are not available, haemodiluted samples can only be accepted if the testing procedures used have been validated for such samples.

b. Previous diagnosis of disease, surgeries, vaccinations, genetic disease, chronic diseases and family history should be evaluated. For living allogeneic donation, where applicable, attention should be given in cases of family adoption or conception by donated gametes/embryos, as it may not be possible to trace the genetic family history. Thorough investigation of the previous diseases of the potential donor must be carried out. The evaluation should include any past medical history related to:

   i. chronic/previous disease, e.g. chronic persistent infection, malignancy, autoimmune disease, neurological disease, genetic disease,
   ii. medication,
   iii. information on recent vaccinations [4]:
      - to identify recent vaccinations that indicate travel risks,
      - to identify vaccinations with live attenuated virus,
      - to help with interpretation of test results (a recent HBV vaccination is expressed as reactive/positive HBs Ag).
   iv. family history, for instance if individuals are at familial risk of prion-associated diseases (have had two or more blood relatives develop a prion-associated disease, or have been informed following genetic counselling that they are at risk for public health purposes) [5], or for malignancies or connective tissue disease.

4.3.1.2.2. Social history, evaluation of behavioural and personal risk

Behavioural and personal risk (including travel history) must be evaluated as they may completely exclude a donor, or indicate that certain tissues/cells may be compromised or suggest an increased risk of infectious diseases [6].

It is necessary to ask about sexual behaviour (e.g. commercial sex workers, frequently changing partners regardless of their gender, men having sex with men, history of sexually transmitted diseases). The use of intravenous drugs and lifestyle should be queried.

Travel history or residence abroad/overseas must be evaluated to rule out the risk of tropical or endemic infections, e.g. malaria, trypanosomiasis or Zika, as well as the subsequent risk of vertical

transmissions. Emerging, non-tropical infections also exist in some European regions, e.g. West Nile virus, chikungunya virus.

Any history of travel or residence abroad must be expanded with information about living conditions, migration background, refugee status and workplaces (e.g. sewage plant, woodlands, farm, airport, hospital). This helps to identify risks related to places/countries with less rigorous regulatory standards or with a high prevalence of certain infections. Information about hobbies (e.g. home, garden, animals, woodlands) should also be obtained with the same intention.

Seeking information about contact with fauna, especially bites from pets, domestic or wild animals, bats and birds, is necessary to evaluate the risk of infections.

4.3.1.3. *Physical evaluation of donors*

Physical evaluation of the donor (see Table 4.2) should be carried out before procurement and must be documented. Each donor (adult or child) must be thoroughly examined following established protocols (see Chapter 6), covering the anterior and posterior aspects of the body as well as an inspection of body cavities. Excessive weight of the donor cannot compromise the requirement to carry out a thorough assessment. The information obtained through physical examination is supplementary to the comprehensive summary of clinical data. Any findings suggestive of possible risk should be investigated.

For living donors, a complete physical examination should be undertaken to ensure the safety of donors and recipients according to the specific requirements of the particular type of tissue or cell donated. This examination should be done in the context of a clinical evaluation that includes an interview and a comprehensive physical examination, together with psychological evaluation of the potential donor. More detailed information about HPC, including paediatric donors and non-partner MAR donors, is given in Chapters 22 and 27.

For deceased donors, the physical examination should look for evidence of high-risk behaviour, or external signs of underlying medical conditions (see Table 4.2). Visual examination of the body is advisable during early, initial screening if adequate information on the condition of the body cannot reliably be obtained orally. The physical examination may include taking a picture of suspicious lesions that may indicate a risk or taking a sample for histology. Any findings that may indicate the risk of transmissible disease or unsatisfactory quality of the tissues should result in exclusion of the donor [7]. Any new information related to lesions (tumours, skin lesions, scars), diseases or treatments that becomes apparent during the physical examination must be investigated further by the professionals responsible for donor selection.

In all cases of abnormal findings, each tissue establishment should establish – following their SOPs – whether further investigations should be carried out. The limited sensitivity and specificity of physical examination for discovering pathologies must be considered in the donor risk assessment. An example of a tissue-donor physical assessment form can be found in Appendix 15.

Because the physical examination can result in rejection of a donor before procurement, or of the tissues or cells after procurement, its importance is clear [7].

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2770 **Table 4.2. Deceased donors- Physical examination prior to donation**

Look for signs of:

a. possible systemic disease:

- malignancy (suspicious skin or subcutaneous lesions; see Appendix 16);
- malnutrition, multiple deformities;

b. bacterial or viral infection:

- recent receipt of a live vaccination (vaccination site infection, scabs, vaccinia;
- recent receipt of a tattoo, body piercing or acupuncture where non-sterile instruments may have been used (shaved area, redness, swelling or scabbing may require further investigation to assess risk);
- skin lesions such as a rash, petechiae, skin ulcers, blue/purple or grey/black lesions, shingles, scabs;
- oral lesions such as ulcers or thrush (not always possible to examine due to rigor mortis);
- enlarged lymph node(s);
- icterus, hepatomegaly;

c. high-risk behaviour

- injected drug abuse (non-medical injection sites);
- inspection of tattoos for hidden injection sites or for any additional information (e.g. some tattoos may suggest imprisonment or high-risk sexual behaviours);
- genital or peri-anal skin lesions indicative of a sexually transmitted disease (e.g. evidence of anal intercourse, herpetic lesions or ulcerative disease);

d. trauma:

- fractures, avulsions, lacerations or abrasions that may affect (contaminate, compromise integrity of) the tissue to be procured;
- internal trauma that can cause cross-contamination between cavities (e.g. injury to the bowel, penetrating or crushing injuries);
- cleanliness of the body, the condition in which the body was found (this can also relate to increased risk for contamination/cross-contamination);
- scars (surgical or other); if findings do not match the donor's history, further investigation may be required.

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2772 ***4.3.1.4.*** *Special considerations for paediatric donors*

2773 Child donors must be screened with as much diligence as adult donors. Physical assessment must not be
2774 overlooked or shortened simply because the donor is a child. Although risk associated with sexual
2775 activity may not seem relevant, infectious disease associated with child abuse (sexual) is possible, so
2776 examination of the genital and peri-anal regions is recommended.

2777 Additional considerations are required for living paediatric donors. For donors under 18, or the
2778 relevant national legal age of consent, the parents or guardian normally give consent, but the minor, if
2779 possible, should assent to the procedure and the screening questions should be tailored to the age of the
2780 minor donor [1].

2781 Special screening considerations are also required for some paediatric donors. An infant's
2782 immune system is not fully developed, so protective antibodies may not yet have been produced against
2783 infection, thereby increasing the risk of undetectable infection with serologic screening. If the child is
2784 18 months old or younger, or has been breastfed in the 12 months before death, the birth mother should
2785 be tested and evaluated for risks associated with HIV, HBV, HCV, HTLV and syphilis, as with any other

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allogeneic donor (see Chapter 5). Other diseases that can be transmitted vertically from mother to foetus may also be relevant, such as malaria or Chagas disease.

In the EU, Directive 2006/17/EC stipulates that children aged under 18 months born to a mother with infection by HIV, HBV, HCV or HTLV, or who are at risk of such infection, and who have been breastfed by their mothers during the previous 12 months, cannot be considered as donors regardless of the results of analytical tests.

4.3.2. Generic contraindications for tissue and cell donation

The guidelines for excluding or including donors presenting certain risks vary between countries and regions and are determined by local disease prevalence and risk assessments. Therefore, this list of risk criteria should be regularly reviewed and modified according to local circumstances, as epidemiological changes and future developments in diagnostics occur.

Despite these limitations, donors should be considered as high-risk if one or more of the following conditions are present.

4.3.2.1. Unknown cause of death (in deceased donors)

If the cause of death (COD) is not known, the donation cannot be permitted, because death may have been due to a disease that could be transmitted to recipients of tissues and cells. The only exception would be in those cases where an autopsy is performed and can clarify the COD after tissue procurement. The circumstances of death and medical history for differential diagnosis contributing to death may help to exclude contraindications to donation until the certified cause of death is available.

4.3.2.2. Infectious diseases

Infectious agents transmissible by organs or tissues belong to one of five groups of pathogens

- **Viruses** by infection in the tissue of donor with or without current viraemia. Thereby DNA-viruses may persist latently in the tissues without detectable viraemia; RNA-viruses usually cause direct infection and disease;
- **Bacteria** by bacteraemia or colonisation/infection of organs or tissues;
- **Fungi** by fungaemia or colonisation/infection of organs or tissues;
- **Parasites** by latent or acute infection;
- **Prion** (see §4.3.2.2.5 and §4.3.2.2.6).

4.3.2.2.1. Active systemic infection

Donors with systemic infection that is not controlled at the time of donation (including bacterial diseases, viral, fungal, protozoan or parasitic infections, or significant local infection in the tissues and cells to be donated) should be excluded. Donors with bacterial septicaemia may be evaluated and considered for (avascular) cornea donation, but only if the corneas are stored by organ culture (see Chapter 16).

If the aetiology of an active infection cannot be established, the donor is not a suitable candidate for donation. Communication with the physician or medical staff caring for the potential donor is necessary if there is any doubt. These healthcare providers may know if there was a suspicion of sepsis or another infectious disease at the time of death, which may not have been well documented in the records.

4.3.2.2.2. Chronic persistent infection

Consider the history of bacterial and protozoic diseases that can lead to chronic persistent infections, including tuberculosis, brucellosis, leprosy, Q fever, chlamydiosis and salmonellosis. Specific attention should be paid to tick/arthropod-borne diseases such as borreliosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis. The risk of transmitting these infectious agents with specific tissues must be assessed, and negative effects for the recipient(s) excluded.

4.3.2.2.3. Proven transmissible viral infection

Donations must be screened for evidence of transmissible viral infections (see Chapter 5). Persons with clinical or laboratory evidence of (i.e. have tested positive for) HIV, HCV, HBV or HTLV-I/II

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2834 infection are excluded from donation. Behavioural risks that could increase the risk of acquiring
2835 transmissible infections are discussed in section 4.3.3.1

2836 *4.3.2.2.4.* Recent history of vaccination with a live attenuated virus/bacterium
2837 Vaccinations with live vaccines [4] may result in transmission of a vaccine-derived pathogen to
2838 the recipient. Therefore, it is imperative to determine if the donor has received live vaccines during the
2839 previous 4 weeks. Live vaccines include:
2840 a. Viral: inhaled attenuated influenza (not injectable, inactivated influenza), varicella–zoster,
2841 rotavirus, measles, mumps, rubella, oral polio (not injectable, inactivated), oral cholera (not
2842 injectable, inactivated *Vibrio cholerae*) and yellow fever. Vaccinia for smallpox should be
2843 deferred for 8 weeks;
2844 b. Bacterial: bacillus Calmette-Guérin (BCG), oral *Salmonella typhi* (not injectable, inactivated).

2845 *4.3.2.2.5.* History of Prion disease
2846 Transmissible spongiform encephalopathies (TSE), which include Creutzfeldt–Jakob disease
2847 (CJD), Gerstmann-Stäussler-Scheinker (GSS), Kuru and fatal familial insomnia (FFI), are rare
2848 neurological degenerative diseases that are progressive and inevitably fatal. They are associated with
2849 transformation of the normal form of prion protein (PrP^{C}) into an abnormally-folded form (PrP^{Sc}).
2850 There are four clinical forms of CJD: sporadic (sCJD), which is the most common; variant
2851 (vCJD); genetic (gCJD), and iatrogenic (iCJD). While Western blot and ELISA assays have been
2852 investigated for testing blood, retinal tissue, optic nerve, spleen and tonsillar tissue, diagnosis can
2853 currently be confirmed only by autopsy. Adherence to European Centre for Disease Prevention and
2854 Control (ECDC) recommendations is suggested and the risk of transmission should be considered as
2855 detailed in 4.3.2.3.

2856 *4.3.2.2.6.* Risk of transmission of prion diseases
2857 TSE transmission risk should be considered in the following cases:
2858 a. persons diagnosed with any form of CJD, GSS or FFI;
2859 b. any suspicion of prion-associated disease, such as rapid progressive dementia;
2860 c. a diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has
2861 been ruled out by microscopic examination). If dementia has a primary cause (e.g. dementia of
2862 vascular origin), donation can be accepted;
2863 d. degenerative or demyelinising disease or a disorder of unknown aetiology involving the central
2864 nervous system;
2865 e. persons treated with hormones derived from human pituitary gland, such as growth hormone;
2866 f. recipients of cornea, sclera and *dura mater* as well as persons who have undergone undocumented
2867 neurosurgery in which the *dura mater* may have been used;
2868 g. persons who lived in the UK between January 1980 and December 1996 for longer than 6 months
2869 (for countries other than UK);
2870 h. individuals who have been told that they may be at increased risk because a recipient of blood or
2871 tissues that they have donated has developed a prion-related disorder [5].

2872 *4.3.2.3. Malignancies*

2873 *4.3.2.3.1.* Haematological malignancies
2874 Myeloid and lymphoid neoplasia and leukaemia are malignant diseases caused by dysregulated
2875 multipotent haematopoietic stem cells and should be considered as absolute contraindications to
2876 donation. Other myeloproliferative diseases may also affect the stem cells; thus, these donors require
2877 special attention, and donation of living cells is not recommended. The major subtypes of myeloid
2878 neoplasm and acute leukaemia, according to the updated World Health Organization (WHO)
2879 classification [8], and WHO classification of lymphoid neoplasms [9] are listed in Appendix 17 (Tables
2880 A&B). Any information indicating haematological alterations that would be suggestive of any of the
2881 malignancies listed in Appendix 17 (Tables A&B) must be evaluated further. A recent blood test carried
2882 out before death, if available, may offer valuable information indicative of these alterations. Although

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an experienced haematologist will be able to provide a differential diagnosis, certain results should be individually evaluated [10], such as the examples shown in Table 4.3.

Table 4.3. Haematological malignancies that should be individually evaluated

Altered haemoglobin	men > 18.5 g/dL women > 16.5 g/dL	should be carefully assessed for potential contraindications, such as *polycythemia vera*
Altered haematocrit	men > 55.5 % women > 49.5 %	
Platelet count	$< 50 \times 10^9$/L	is highly indicative of a haematological disorder
	50-100 $\times 10^9$/L	should be carefully assessed for contraindications due to a possible haematological problem
	$> 450 \times 10^9$/L	should be carefully assessed for contraindications such as essential thrombocytosis
Altered white blood cells	$> 50 \times 10^9$/L	should be carefully assessed for contraindications such as chronic myeloid leukaemia

4.3.2.3.2 Non-haematological malignancies

A history of malignancy should be evaluated carefully to determine its effects on the quality and safety of tissue, because of either the presence of a tumour or the treatment given to the donor for malignancy.

Results of donor evaluation may imply a donor risk, a recipient risk or both. An increased risk of harm to a living donor is not acceptable, even if the benefit of transplantation for the recipient is considered to outweigh the risk of transmission (for example in the case of past malignancies).

Detailed history of type, duration, course/recurrence and treatment history must be considered. Availability of screening programmes has improved early detection, which increases the treatment options and can lead to cure. For donors who had been diagnosed with a pre-malignant condition (e.g. Bowen's disease, polyposis coli or Barrett's oesophagus), further information should be sought to exclude malignancy because these patients are likely to be monitored regularly.

Some international bodies provide assessments on risk of transmission of malignancies through organ transplant for CNS (central nervous system) and non-CNS tumours [11], which can be used as a basis for determination of the risks of transmission through tissue transplant. The role of processing steps applied to tissues and cells in reducing the risk of transmission of malignancy for tissues and cells following transplantation should be considered. The effect of high-dose terminal sterilisation (25-40 KGy) and the decellularisation process removing viable cells are examples of tissue-processing steps that reduce the potential for transmission of malignant cells.

The *Guide to the quality and safety of organs for transplantation* (Chapter 9: risk of transmission of neoplastic disease) [12] provides useful guidance on assessing the risk of malignancy transmission through organ transplantation, based on published evidence in the literature and national transplant registries. In addition to absolute contraindications (unacceptable risk), the risk of transmission is classed as minimal (< 0.1 %), low (0.1 to 1 or < 2 %), intermediate (1 or 2 % to 10 %) and high (> 10 %) for CNS and non-CNS tumours. A similar approach could be applied for tissue and cell transplantation based on literature review.

4.3.2.3.3. CNS tumours

Malignancy gradation in the CNS should be thoroughly evaluated, including a complete histological exam rather than a simple biopsy, due to possible heterogeneity of the mass. The 2016 WHO classification [13] of selected CNS tumours is shown in Appendix 17 (Table C). WHO Grade I and II CNS tumours have a minimal or low risk of metastasis and would not normally contraindicate tissue donation. The Grade III and IV primary CNS tumours have either low (WHO grade III: < 2 % transmission risk) or intermediate (WHO grade IV: 2.2 % with an upper 95 % CI of 6.4 % transmission risk) risk of transmission through organ transplantation [11] (Appendix 17 Table D). In CNS tumours,

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cerebral lymphoma and secondary intracranial lymphomas are considered absolute contraindications. Individual risk assessment is required for decision making for accepting donors with malignancies that have low and intermediate transmission risk for tissue donation.

4.3.2.3.4. Non-CNS tumours

The assessment of transmission risk for non-CNS tumours in an organ transplant setting [11] is summarised in Appendix 17 Table E. Malignant neoplasms have been transmitted to immuno-suppressed recipients through transplantation organs from donors with known or unknown malignancies. In an organ transplant setting, this risk needs to be considered against the perspective of the important, life-enhancing and life-saving benefits afforded by organ transplant. The increasing number of patients on waiting lists, along with the shortage of organs available for transplant, has encouraged reconsideration of the criteria for acceptance of organs from donors with a past or current history of malignancy [12].

The risk–benefit analysis is very different in a tissue-transplant setting. The tissue grafts are mostly used in elective settings and are life-enhancing (rather than life-saving). There may be other suitable bioprosthetic grafts. On the other hand, unlike organ recipients, tissue recipients usually do not require immuno-suppression. The processing steps and terminal sterilisation may vastly reduce the potential for transmission of tumour cells. The risk of transmission is generally much lower compared with organ transplant. There have been two recent case reports (2017) of donor-derived malignancy in keratolimbal allograft recipients [14, 15].

4.3.2.3.5 Carcinoma *in situ* (CIS)

CIS is an early form of cancer that is defined by the absence of invasion of tumour cells into the surrounding tissue, usually before penetration through the basement membrane. CIS is, by definition, a localised phenomenon, with no potential for metastasis unless it progresses into an invasive carcinoma. Therefore, its removal eliminates the risk of subsequent progression into a life-threatening condition. When explaining a laboratory report to a patient, most doctors will refer to CIS as “pre-cancer”, not cancer. In the TNM classification, CIS is reported as TisN0M0 (Stage 0).

Because most forms of CIS have a high probability of progression into invasive carcinoma [12], it is usually recommended that the lesion be completely removed. Therefore, CIS is usually treated in much the same way as a malignant tumour. If a donor had been successfully treated and cured after a CIS (e.g. cervical or vulval carcinoma *in situ*, some intra-ductile carcinoma of the breast, intraepithelial cancer of the prostate etc.,) the donation could be suitable because CIS do not adversely affect the safety or quality of other types of tissues like *in situ* carcinoma of the cervix

4.3.2.3.6. Specific considerations for EU member states

It must be noted that, for EU member states, the EU directives for tissues and cells general donor exclusion criteria requires that donors with malignancy must be excluded from donation unless justified on the basis of a documented risk assessment approved by the responsible person (as specified below). Commission Directive 2006/17/EC states that the presence, or previous history, of malignant disease, except for primary basal cell carcinoma, carcinoma *in situ* of the uterine cervix and some primary tumours of the central nervous system that have to be evaluated according to scientific evidence, is a criterion for exclusion of donors for tissue or cells. This regulatory requirement must be considered as part of the risk assessment in decision making.

Donors with malignant diseases can be evaluated and considered for cornea donation (see Chapter 17), except for retinoblastoma, haematological neoplasm and malignant tumours of the anterior segment of the eye. Malignant melanoma with known metastatic disease also excludes use of ocular tissue, including avascular cornea. Any vascularised ocular tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and should be evaluated as discussed above.

4.3.2.4. Exposure to toxic substances

In case of ingestion or exposure to a toxic substance (e.g. cyanide, lead, mercury, gold, arsenic, pesticides), the quality and safety (due to the presence of high level of substance) of some types of tissues and cells may be affected and, as a result, can cause harm to recipients. Exposure to asbestos in

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2971 the past is a risk for developing of a mesothelioma. In this case a thorough risk assessment should be
2972 performed to estimate this risk.

2973 *4.3.2.5. Tissue-specific contraindications*

2974 Certain medical conditions can adversely affect specific tissues and cells which, if procured,
2975 processed and made available for human application, may result in unfavourable outcomes for the
2976 recipients of tissue and cells. This risk is evaluated on a case-by-case basis and for specific tissue types.
2977 For guidance on the specific contraindications for each tissue and cell type, please refer to the relevant
2978 chapters in Part B of this Guide.

2979 **4.3.3. Evaluation of personal and behavioural risk**

2980 All substances of human origin (SoHO) have the potential to transmit infections to a recipient.
2981 Behavioural risk is evaluated to inform assessment of donor suitability. Evidence-based donor selection
2982 is the first safeguard in minimising the risk of transmission while not compromising sufficiency of
2983 valuable grafts for clinical use.

2984 The incidence and prevalence of these SoHO-related infections varies, depending on different risk
2985 factors [16, 17, 18], and the causes of *de novo* infection vary between European regions [16, 19]. The
2986 tissue establishment must consider available evidence from the epidemiological data on transmissible
2987 blood-borne infections such as HIV, HCV and HBV in the population, the performance (sensitivity and
2988 specificity) of screening tests used for detecting these infections and the residual risk of undetected
2989 infection that could be potentially transmitted to the recipient. This residual risk may be the result of one
2990 or all of a number of factors: error in the process, poor assay sensitivity, a donation collected from a
2991 donor in the infection window period. International peers adopt an interval of at least twice the window
2992 period since the last "at-risk behaviour" for the length of deferral before donation [16].

2993 It is recommended that a risk-assessment framework, such as the Alliance of Blood Operators
2994 model [20], is used to systematically analyse the information and document the decision, based on the
2995 acceptable level of risk tolerance. The outcome of this systematic approach would provide the basis for
2996 evidence-based donor deferral and acceptance policies for donors with high-risk behaviours and their
2997 sexual partners [21].

2998 *4.3.3.1. Behavioural risk factors*

2999 Potential donors should be considered at high risk if they have participated in any of the following
3000 behaviours or if they have had sexual contact with persons who have participated in any of the following
3001 behaviours:

3002 i. People who have injected drugs by an intravenous, intramuscular or subcutaneous route for non-
3003 medical reasons;
3004 ii. Tattoos, ear piercings, body piercings and/or acupuncture, which are very popular in some
3005 European countries; usually they are applied by sterile methods and in many countries, there are
3006 specific approvals for those establishments. If tattoos, piercings or acupuncture were done in
3007 approved settings, the donor can be accepted without temporary deferral, but in case of doubt the
3008 associated risk should be considered similar to that of non-medical injections;
3009 iii. Persons who have been newly diagnosed with, or have been treated for, sexually transmitted
3010 diseases (e.g. syphilis, gonorrhea, *chlamydia* or genital ulcers);
3011 iv. Men who have had sex with men (MSM);
3012 v. Persons who have had sex in exchange for money or drugs;
3013 vi. Persons whose sexual behaviour, including frequent changes of sexual partner, puts them at risk
3014 of acquiring severe infectious diseases.

3015 In countries where tissue establishments do not have access to data to perform risk assessment, a
3016 deferral period of 12 months is a safer option after cessation of the high-risk behaviour or sexual contact.
3017 This may be reduced to 3 months if supported by risk assessment, considering risks and benefits of the
3018 transplant, together with single NAT testing and bacterial screening.

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3019 *4.3.3.2. Personal risks, exposure events [5]*
3020 Exposure events that increase the risk of acquiring a communicable disease can occur at any time
3021 during life. They include accidents, certain medical therapies, occupations and travel to, or residence in,
3022 an area endemic for certain diseases. Here are examples of other risk factors:
3023 i. Persons from a high-risk region for endemic disease, e.g. HIV-1 group O, human T-cell
3024 lymphotropic virus (HTLV-I). The Caribbean is, for example, high-risk for HTLV-I;
3025 ii. Exposure to someone else's blood (such as needlestick injury, human bite) when that person was
3026 known to be infected with HIV, HBV or HCV;
3027 iii. Sharing a residence with someone who has HBV or clinically-active HCV;
3028 iv. Persons regularly transfused with blood or blood products should be carefully evaluated case by
3029 case for the risk of disease transmission;
3030 v. Persons with haemophilia or related clotting disorders who received human-derived clotting factor
3031 concentrates before 1987, in a period when more advanced methods for manufacturing those
3032 products were not widely used;
3033 vi. Patients with chronic haemodialysis;
3034 vii. People who have been in a lockup, jail, prison or juvenile correctional facility for more than 72
3035 consecutive hours should be carefully evaluated for the risk of high-risk behaviours (see 4.3.3.1);
3036 viii. A history of travel to, origin in or visiting relatives in malaria-endemic areas;
3037 *The minimum deferral period recommended for blood donors in EDQM guidance [19] for **all***
3038 *groups of potential donors (visitor, origin/previous resident, travel-related illness, history of*
3039 *malaria) is 4 months if the result of a validated malaria antibody assay, performed at least 4-*
3040 *months after last exposure incident or resolution of symptoms, is negative.*
3041 ix. A bite from an animal suspected of having rabies at any time;

3042 *4.3.3.3. New and emerging diseases*
3043 New and emerging diseases (including those that have spread to a new geographical area) can
3044 pose a significant challenge when screening donors for risks of communicable disease due to travel
3045 history. Professionals responsible for donor selection should be vigilant regarding surveillance of
3046 changes to the global movement of infectious-disease risks. Diseases that should be considered include:
3047 Middle East respiratory syndrome (MERS), dengue fever, yellow fever, malaria, trypanosomiasis,
3048 tuberculosis, plague, chikungunya virus, West Nile virus (WNV), severe acute respiratory syndrome-
3049 associated coronavirus (SARS-CoV), Q fever, antibiotic-resistant diseases, and HIV-1 group O, rabies,
3050 Ebola virus and Zika virus. In Europe, regular monitoring of the Rapid Communication Reports
3051 originating from the Eurosurveillance website [22] is recommended, as well as actively seeking
3052 information to assess the epidemiological status of diseases in the areas where a donor has lived or
3053 travelled [23]. Specific information about geographic distribution of infectious diseases can be obtained
3054 from the websites of the European Centre for Disease Prevention and Control (www.ecdc.europa.eu),
3055 the World Health Organization (www.who.int/ith/en) and the Centers for Disease Control and Prevention
3056 in Atlanta (the Yellow Book at wwwnc.cdc.gov/travel).
3057 The risk of transmission of an infectious agent through procurement of tissues or cells from a
3058 donor who may have visited an affected area should be balanced against the likelihood of transmission
3059 occurring. Regional risks within an affected country can vary. In cases of recent travel, if the donor
3060 remains well after return or after known contact with someone infected, the donor should be deferred
3061 for at least twice the length of the incubation period [17]. If the donor was infected, they can only be
3062 accepted after full recovery and when the donation is no longer infectious.

3063 **4.3.2. Relative contraindications**
3064 Below are listed the potential risks that have to be analysed on an individual basis, considering
3065 the potential harm and benefit.
3066 a. Additional contraindications/risks to donation of tissues and cells for living donors:
3067 i. pregnancy (except for donors of umbilical cord blood cells and amniotic membrane, and sibling
3068 donors of haematopoietic progenitor cells);

3069 ii. breastfeeding;
3070 iii. health risks for donors themselves (e.g. specific procedure or superovulation).
3071 b. Organ-transplant recipients:
3072 Organ recipients receive immuno-suppressive drugs to prevent rejection, but this could make the
3073 serology testing unreliable; moreover, organ donor-selection criteria are less stringent than for
3074 tissues and cells. This risk should be assessed on a case-by-case basis, taking into account the
3075 level of immuno-suppression in combination with the possibility of tracing the medical details
3076 of the organ donor.
3077 c. Impact of immuno-suppressive agents in the donor:
3078 Treatment with immuno-suppressive agents can weaken the immune system and thus influence
3079 the reliability of serological tests (Chapter 5). Evaluating the effect of the immuno-suppressive
3080 agents on the haematological parameters (erythrocytes, leukocytes and thrombocytes) can be
3081 indicative for immuno-suppression. NAT testing may be helpful in such circumstances. All
3082 other medication of the donor should be always interpreted by a risk assessment for impact on
3083 the tissue, e.g. chronic use of corticosteroids can affect the quality of skin and musculoskeletal
3084 tissue (see Part B: tissue-specific chapters).
3085 d. History of genetic disease:
3086 A family history of genetic disease is a risk factor that should be assessed; where the occurrence
3087 of genetic disease in the family history cannot be traced/assured, this increases the risk of
3088 transmission of genetic disorders, especially in non-partner MAR (see Chapter 27), and should
3089 be regarded as an exclusion criterion (see Chapter 22 for HPC).
3090 e. Deferred for blood donation for known reason:
3091 If it is known that the potential donor was excluded or deferred from donating blood by a blood-
3092 collection establishment, the specific reason for the deferral must be discovered, and the
3093 eligibility of the donor is then evaluated on an individual basis. If the reason is not known, it
3094 may be safer to exclude the donor for tissue donation.
3095 f. Xenotransplantation [24, 25, 26, 27]:
3096 The reference to 'transplantation with xenografts' is clearly stated as an exclusion criterion for
3097 donors at **§1.1.13** of Annex I in Directive 2006/17/EC. However, the absence of a formalised
3098 definition for its interpretation has previously led to some ambiguity in its application. Similar
3099 terms – such as 'xenotransplantation product', 'xenogeneic cell-based medicinal product' and
3100 others – have been used within different healthcare sectors, and the need for a uniform consensus
3101 on terminology is paramount. In recent years different Scientific and Technical Committees have
3102 established and adopted the fundamental opinion that the term 'xenotransplantation' is applicable
3103 to any procedure that involves the transplantation, implantation or infusion into a human recipient
3104 of either (a) live cells, tissues or organs from a non-human animal source, or (b) human body
3105 fluids, cells, tissues or organs that have had *ex vivo* contact with live non-human animal cells,
3106 tissues or organs.
3107 The scientific community continues to apply the principles of this approach, and
3108 xenotransplantation products include those which utilise living non-human animal cell, tissues or
3109 organs used for transplantation. With similar equivalence, the US Guidance for Industry has
3110 adopted the scientific opinion and further states that any biological products, drug or medical
3111 devices sourced from non-living cells, tissues or organs from non-human animals are not
3112 considered xenotransplantation products (e.g. porcine insulin and biological heart valves). The
3113 risk-management strategy for the control of infectious agents is primarily focused on the
3114 application of selective sourcing, effective collection and handling, and measures applied for
3115 elimination/inactivation or removal of agents. Where relevant, the medical device and medicinal
3116 sectors are regulated by these standards to achieve high standards of quality and safety, and the
3117 products are thus viewed as risk-mitigated.
3118 As a precautionary measure, a few countries have applied a broader interpretation of the
3119 exclusionary term, to also include non-viable cells or tissues of animal origin utilised in

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therapeutic products. Tissue establishments should apply documented systems to justify their local practices in relation to xenotransplantation products by the evaluation of scientific evidence, professional standards and national guidance.

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3195 **Related documents:**
3196 **Appendix 14. Sample donor assessment form;**
3197 **Appendix 15. Sample donor physical assessment form;**
3198 **Appendix 16. Practical guidance for the evaluation of pigmented skin lesions and differential diagnosis of**
3199 **melanoma**
3200 **Appendix 17. Evaluation of malignancies for risk assessment in tissue donors**
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Chapter 5. Donor testing: markers for infectious diseases

5.1. Introduction

Use of tissues and cells for human application can result in unintentional transmission of disease. However, such events can be prevented by scrupulous evaluation of donors, including laboratory testing of each donor close to the time of donation in accordance with established good practice. The risk can be reduced substantially by appropriate donor sample testing, but adequate controls must be in place to ensure that test results are accurate and reliable. Controls include:

a. ensuring that the screening programme includes notably relevant infectious diseases (related to the habitus and travels, if any) and their appropriate individual screening target(s);
b. selecting a suitable testing laboratory;
c. ensuring that donor blood samples are labelled, handled and stored appropriately and that the time interval between collection and testing meets the sample requirements of the test kit;
d. use of appropriately validated tests for infectious diseases;
e. providing well-written standard operating procedures (SOPs) and training for all personnel involved in collection and labelling of donor samples, for sample storage and transport, and for technical staff carrying out testing and reporting results, as well as for those receiving and interpreting them.

These are vital elements of a tissue establishment's quality system, and any laboratory undertaking tissue-donor testing must ensure that the most appropriate technology for this activity is used, including selecting test kits that demonstrate high clinical and analytical sensitivity and specificity.

5.2. General concepts

Tissue establishments must ensure that donor samples from all donations of human tissues and cells are subjected to biological tests mandated by national or other applicable legislation, or by a specific situation such as travel. In EU member states, Annex II of Directive 2006/17/EC, amended by Directive 2012/39/EU, specifies mandatory laboratory tests and general testing requirements for living and deceased donors of tissues and cells, and requires that any such laboratory and its tests must be authorised by the competent authority.

SOPs that define the criteria for acceptance or rejection of tissues and cells based on those test results must be in place. The Responsible Person (RP) who will interpret test results should be knowledgeable about infectious-disease test kits, and decisions must meet the expectations in regulations or, if regulations are not prescriptive, follow professional standards of practice [1]. In EU member states the RP is defined by Article 17 of Directive 2004/23/EC.

Documented measures must be taken by tissue establishments that receive tissues or cells from another country or distribute tissue or cells to another country to ensure that the donor-testing requirements of the destination country are met. Evidence should also be available to show that any laboratory involved in testing of donor samples has been accredited, designated, licensed and/or authorised by the appropriate authority to carry out such testing.

5.3. Quality of donor samples

Manufacturers of assays for infectious-disease testing provide specific sample requirements for which their assays have been validated. Personnel of procurement organisations and tissue establishments involved in collecting, transporting (having particular regard to packaging, temperature, duration), storing or testing donor samples must be aware of these requirements to ensure optimal assay

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3245 performance. If inadequate or otherwise compromised samples are provided to the laboratory and tested,
3246 the results may not be valid, which increases the risk of donor-related transmission of infectious
3247 pathogens.
3248 Donor-related conditions that could affect the quality of a test specimen must also be taken into
3249 account. Infectious-disease test results may be invalidated by haemodilution if the extent of any dilution
3250 is such that it may dilute any screening target present to a level below that which is detectable by the
3251 assay(s) used. Therefore in cases where haemodilution is known to have occurred, ideally pre-
3252 transfusion/infusion samples should be obtained for testing purposes. (See §5.3.2)
3253 Haemolysis may also affect test results. Haemolysis is the destruction of red blood cells in whole
3254 blood that discolours the plasma or serum, and it is noticeable after centrifuging the tube. Depending on
3255 the degree (severity) of haemolysis, the colour of the serum or plasma may be pink to red. This darker
3256 colour can promote a higher density reading by the optical component of test equipment, leading to a
3257 positive test result. Haemolysis may be caused by rapid collection of blood through a small-bore needle,
3258 or by improper sample storage or transport, such as allowing the tube of whole blood to freeze prior to
3259 testing. Other causes can be donor-derived and include an infection, a toxin, medication or autoimmune
3260 haemolytic anaemia, and haemolysis can occur after haemodialysis or after a haemolytic transfusion
3261 reaction.
3262 Although serology tests must be used, detection of antibodies against pathogens can be impaired
3263 if the donor has received immuno-suppressive treatment prior to sample collection or when the donor
3264 has received blood products containing antibodies against Epstein–Barr virus, *Cytomegalovirus*,
3265 *Toxoplasma* or hepatitis B (HBs antibodies). In both cases this leads to problematic test results. In the
3266 first case, that leads to a false-negative result; in the second case, it leads to a false-positive result due
3267 to passive immunity communicated by antibodies included in the validated and authorised labile blood
3268 product. In the first situation, adding molecular screening tests (i.e. nucleic acid amplification
3269 technique/NAT) can be valuable because detection of viral nucleic acid in blood samples is generally
3270 not affected by immuno-suppressive therapy [1, 2]. The underlying condition requiring immuno-
3271 suppression will demand further assessment because the disease/condition in itself may constitute an
3272 independent reason for determining that the donor is not eligible. If any of these donor-related conditions
3273 exist, they must be documented in the donor record and evaluated by an RP before release of tissues or
3274 cells for clinical application. In the second situation, pre-transfusion/infusion samples should be used.
3275 Additionally, false-negative results can occur in different scenarios: haemodilution, incorrect
3276 sampling or inappropriate test quality.

3277 **5.3.1. Sample collection (sample type, tubes, labelling, time limits and handling)**

3278 All personnel involved in any stage of the testing process must be fully trained. Testing must be
3279 carried out on plasma or serum of the donor according to the specification laid out by the manufacturer
3280 of the test kit. Testing must not be performed on other fluids or secretions, such as aqueous or vitreous
3281 humour, unless the assays selected have been specifically validated for use with that sample type. In the
3282 case of a neonatal donor (i.e. age ≤ 1 month), the required tests should be carried out using a blood
3283 sample from the donor's birth mother. Another important consideration is that, during the first 18 months
3284 of life, a child's immune system is only in development and protective antibodies may not yet have been
3285 produced against an infection, thereby increasing the risk of hidden infections in child donors. (See
3286 §5.5.1)
3287 Donor sample collection and manufacturer's test instructions must be followed with regard to:
3288 a. the type of sample collection tube (no anti-coagulant or a specific anti-coagulant) required for the
3289 test being carried out;
3290 b. sample storage and transport conditions post-collection, which can include centrifugation and/or
3291 separation within time limits or specimen refrigeration/freezing; and
3292 c. testing required to be carried out within a specified timeframe post-collection.
3293 To ensure traceability at each stage of the testing process, all donor samples must be identified
3294 with a permanently affixed label that contains information or references that link the sample and the

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laboratory test results to the donor (see Chapters 14 and 15). The date and time when the sample was drawn must be accurately documented. It is recommended that at least two donor identifiers, such as the donor's full name, date of birth and/or medical record number, be used. In the case of a sample from a deceased donor, the label or associated documentation should also include some identification of the person who collected it and a description of the site on the donor's body the sample was taken from (e.g. cephalic vein, femoral artery, subclavian artery, superior vena cava). It is good practice for the identity of all donor samples to be confirmed by a second person (from the procurement team, if possible), and this confirmation process should be documented [3]. If any donor blood samples were drawn before death, they can be validated for use, but there must be assurance that the patient identifier (i.e., appropriate labelling) used for any such specimen is confirmed as coming from the donor so mix-ups do not occur (i.e. to avoid carrying out testing for critical communicable diseases on the wrong person) [3]. Other donor-identification methods can be used, if validated, to ensure traceability [4].

Specimens of blood, serum or plasma from the same donor must not be mixed together for testing, whether collected at the same time or at a different time.

For obvious safety reasons, the collection of donor blood for infectious-disease testing must always occur as close as possible to the donation event. Personnel who collect, or otherwise obtain, donor blood samples to be used for this critical testing must consider factors that could influence sample degradation and cause false-negative or false-positive test results, e.g. time of sample collection, temporary storage conditions.

An adequate volume of whole blood must be collected, because otherwise the quantity of serum or plasma after centrifugation may not be sufficient to undertake all of the required tests for infectious diseases, or for any further investigations that may be required. The volume of blood required will be dependent upon the minimum requirements of the testing laboratories as well as the sample requirements of each test kit, and these parameters should be known and evaluated before blood collection. Other considerations could include a donor with a high haematocrit (which could necessitate collection of extra tubes) and a donor who took (or was given) anti-coagulant medication (fibrin clots may appear in the serum after centrifugation and cause a reduction in the volume of testable serum). If the volume needed is not clear to personnel collecting blood samples from a donor, filling three or four 6 mL blood tubes to their limit should be sufficient. However, in the case of a living donor, care should be taken not to collect an unnecessarily large volume of blood because an adverse clinical event could result. To avoid unintended consequences, personnel who collect donor blood samples should be familiar with the requirements of the testing laboratory, and written procedures should provide specific direction.

Proper handling of any donor blood sample after it is collected is necessary to ensure that testing protocols can meet the required specifications. For example, when a blood sample is collected in a tube containing an anti-coagulant, this liquid or powder requires that a completely filled tube be gently mixed by slowly inverting the tube 5 to 10 times immediately after collection [5].

After collection, specimen handling by personnel can include centrifugation and/or separation of the serum or plasma from red cells within specific time limits. In addition, specimen storage and/or transport conditions can involve refrigerating or freezing the plasma or serum aliquot. Specific instructions from the test kit manufacturer must be followed and can differ between tests [3]. In all cases, validated transport containers and validated shipping conditions must be used when sending donor samples to a testing laboratory.

The facility receiving any donor sample for testing should have an SOP in place to define the criteria for acceptance or rejection of the sample, based on collection, storage and transport conditions. The testing facility must document acceptance or rejection of the sample and should share this sample status in a timely manner with an RP at the procurement organisation or tissue establishment.

5.3.1.1. Deceased donor

In the case of a deceased donor, blood samples must have been obtained just before death or, if this was not possible, the time of sampling must be as soon as possible after death, and in any case within 24 h after death.

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It is important to collect blood samples without untoward delay after death to avoid sample characteristics that could cause a non-specific test result (e.g. partial haemolysis) or that could lead to its rejection for testing (e.g. complete haemolysis). Delays in donor sampling have been shown to increase the incidence of red cell haemolysis, and other substances can appear in non-circulating blood due to growth of micro-organisms and release of enzymes (including by-products of tissue and cell death) [3].

Some studies have demonstrated the potential for blood sample collection to occur more than 24 h after death; however, validation of each infectious-disease test kit using such specimens is necessary to support an extension [6, 7, 8, 9, 10]; see also Appendix 19. Acceptance of such practice is controlled by national regulations.

5.3.1.2. Living donor

In the case of living donors, blood sampling should be obtained at the time of donation or, if this is not possible, within 7 days before or 7 days after donation. However, for practical reasons, collection of a sample from an allogeneic bone-marrow stem cell or peripheral blood stem-cell donor must occur within 30 days before donation (taking into account that re-testing at the time of donation will be informative), but before reaching a point-of-no-return when irreversible measures for preconditioning of the recipient have been initiated. If tissues and cells of allogeneic living donors can be stored for long periods before use, repeat sampling and testing is required after 180 days, unless specific exemption criteria are met. (See §5.5.2; see also Chapters 18-23 and 26-28).

5.3.2. Haemodilution assessment

When possible, a donor blood sample collected before administration of any transfusions and infusions should be used for testing purposes.

If a donor has recently received transfusions of blood or blood components, or infusions of colloids or crystalloids, and has lost blood, any testing of donor blood collected post-transfusion or post-infusion may not be valid due to haemodilution or plasma dilution of the donor's blood and, thus, of any samples taken from the donor. Assessment of the extent of any haemodilution includes the use of a formula to calculate dilution of the donor's original circulating blood volume (and circulating levels of antigen and/or antibody, if present) and should be done by the physician in charge or the transplant co-ordinator. Current practice in a number of countries is to consider 50 % calculated haemodilution to be the maximum allowable with minimal risk of a false-negative test result arising because of the haemodilution.

Examples of when a haemodilution calculation may need to be carried out include:

- *ante mortem* blood sample collection: if blood, blood components and/or colloids were administered in the 48 h preceding blood sampling, or if crystalloids were infused in the hour preceding blood sampling;
- *post mortem* blood sample collection: if blood, blood components and/or colloids were administered in the 48 h preceding death (circulatory arrest), or if crystalloids were infused in the hour preceding death (circulatory arrest).

Refer to Appendix 18 for an example of a commonly used formula to assess the donor's potential haemodilution or plasma dilution that can be applied when the donor received any fluids that may lead to haemodilution. Adaptations of the algorithms may be needed for body sizes outside the normal adult range. Allowances may need to be made for a very large or a very small adult donor, or a paediatric donor. In brief, a donor's total plasma volume (TPV) and total blood volume (TBV) are estimated by calculations based on the donor's body weight, then direct comparisons are made to amounts of recent transfusions and/or infusions that were administered before circulatory arrest or before collection of the blood sample, whichever occurs first [3]:

a. estimate TPV of donor (weight in kg × 40 mL/kg; or, weight in kg ÷ 0.025);
b. estimate TBV of donor (weight in kg × 70 mL/kg; or, weight in kg ÷ 0.015);
c. calculate total blood (mL) received in the last 48 h (A);

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d. calculate colloids (mL) received in last 48 h (B);
e. calculate crystalloids (mL) received in the last 1 h (C);
f. add B + C and compare to TPV (fluid volumes are compared);
g. add A + B + C and compare to TBV (mass/fluid volumes are compared);
h. does either comparison show > 50 % dilution? If not, the blood sample qualifies and can be used for testing for infectious diseases.

Although not normal practice, a tissue establishment may accept tissues and cells from a donor with plasma dilution of > 50 %, but only if each required test has been validated appropriately for use with a diluted test specimen. In such cases, to help reduce risk, additional testing should also be performed using molecular tests (i.e. NAT) for the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), and possibly for other viruses, depending on the donor's travel history, underlying disease or other factors.

The blood collected can also be diluted if the specimen is drawn in close proximity to an infusion or transfusion intravenous line, even if the donor is not actually haemodiluted or plasma-diluted. Samples should be drawn from the opposite side of the body in relation to the site of any infusion/transfusion.

Furthermore, in theory, a transfusion shortly before the donation can result in transmission of infectious agents to the donor.

5.4. Testing laboratories

To meet quality and safety requirements, all testing of infectious diseases for deceased and living donors must be carried out by laboratories that are accredited, designated, authorised and/or licensed for these activities according to the regulations set by the relevant Health Authority. Such laboratories will have the knowledge, skills, resources and competence required for testing blood samples from tissue donors, and should use appropriate algorithms to ensure that their testing procedures have maximum sensitivity without loss of specificity. They must also participate in relevant external quality assessment schemes (proficiency testing) and be subject to regular internal and external audits.

If additional biological assays are carried out, the laboratory used should be accredited and should participate in an appropriate external quality-assessment programme [5]. (See §5.5.1 and Chapter 2.)

Tissue establishments can undertake these testing protocols themselves or have a written agreement with any laboratory that carries out these tests [11]. Tissue establishments should evaluate and select a testing laboratory on the basis of its ability to generate reliable and appropriate results, and to keep relevant records. In addition the testing laboratory must comply with regulatory requirements and any other specific expectations of the tissue establishment (e.g. time-sensitive availability of test results, record retention). The tissue establishment should ensure that the laboratory is competent to perform this work and is using appropriate assays and procedures (ideally, with kit designed for donor screening rather than for diagnosis). There must be evidence that good laboratory practice is being followed and that personnel are appropriately trained and experienced in relevant testing procedures. The obligation of the laboratory to notify the tissue establishment when various deviations occur is mandatory. To ensure a consistent level of competence and performance, audits of the testing laboratory(ies) should be undertaken periodically by the tissue establishment or by qualified external experts as part of the tissue establishment's quality system.

In addition, test records at the laboratory must be retained for 10 years at least and must contain the date of receipt of the blood sample at the testing facility, a record of each test kit used to test donor blood samples (i.e. manufacturer, lot number, expiry date) and the results of donor testing, including repeat testing (if applicable). See Chapters 2 and 15.

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5.5. Tests to be carried out

The donor-screening assays selected must be validated and used in accordance with current scientific knowledge. A higher test generation (e.g. 4th generation) leads in general to a shortened serological window period [12, 13, 14].

All assays used for donor testing within the EU should be Conformité Européenne (CE)-marked (see Appendix 19). Most of the major international manufacturers of donor-screening assays provide CE-marked assays and systems, and in some cases the manufacturers have undertaken validation work with samples from deceased persons. Where such data are not provided, laboratories performing this work will be expected either to have validated the assays for this purpose themselves or to use other available peer-reviewed data [15, 16].

5.5.1. Mandatory tests

Mandatory serological tests for HBV, HCV, HIV and *Treponema pallidum* are listed below; additional molecular assays can be carried out to confirm a putative infection. All assays must be carried out in serum and/or plasma samples of the donor according to the manufacturer's instructions [3]. Those tests, depending upon the laws of the relevant country, must/should be performed in individual samples, not in pooled samples.

a. Human immunodeficiency virus type 1 and 2:
A combination assay (4th generation) including detection of **anti-HIV-1/2 antibodies plus HIV-1 p24 antigen** is strongly recommended. If a 3rd generation test is used, an HIV-1 RNA test (qualitative or quantitative) has to be performed additionally to exclude an HIV-1 infection. It is recommended that the sensitivity limit for the HIV-1 RNA assay should be ≤ 50 IU/mL. Samples with confirmed non-negative (i.e., weak reactive or borderline) serological screening results can be re-tested in duplicate using the same assay. If the results are negative the donated tissues can be released. Reactive samples can be re-tested using an alternative certified serological assay of equal or greater sensitivity. Donations that are non-reactive in that assay and negative for HIV-1 RNA can be considered suitable for clinical use.

b. Hepatitis B virus:
HBV surface antigen (HBsAg) and total antibodies to **HBV core antigen (anti-HBc)**. HBsAg must be negative. If anti-HBc is "reactive", an additional determination of a highly sensitive HBV-DNA method must be performed (currently ≤ 12 IU/mL detection limit); but haemodilution may influence the limit of detection. If anti-HBc is positive and HBsAg and HBV-NAT is negative, the donated tissues can be released. HBV-DNA positivity reflects potential infectivity and leads to a discard of the donated tissues.

c. Hepatitis C virus:
The screening for an HCV infection is based on detection of anti-HCV antibodies. But combined assays like the assays for HIV are not commercially available at present, and the performance of HCV-antigen assays is not yet sufficient to exclude an early infection. Presence of anti-HCV may indicate an acute, chronic or past infection. Furthermore, the pre-seroconversion window phase takes several weeks; thus, an **HCV-RNA assay** is strongly recommended to exclude active HCV infection. It is recommended that the sensitivity limit for the HCV-RNA assay should be ≤50 IU/mL. An anti-HCV-positive and RNA-negative result, which is indicative of a non-specific reaction or a past infection (confirmed from the donor's medical history, i.e. type and duration of HCV treatment and serology), needs to be confirmed by immunoblot analysis. If the result of the HCV immunoblot is negative (anti-HCV false-positive result) the donated tissues can be released. In the case of an HCV immunoblot positive result (confirmed positive anti-HCV result), only with the evidence for successful HCV treatment under medical supervision and a negative HCV-RNA test, the donated tissues can be released as well.

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3488 d. *Treponema pallidum:*

3489 In the serological diagnosis of syphilis, a **treponemal screening test** should be used, e.g.
3490 *T. pallidum* haemagglutination (TPHA) test, the *T. pallidum* particle agglutination (TPPA) test,
3491 Treponemal enzyme immuno-assays (EIA) or chemiluminescence immuno-assays (CLIA). If the
3492 screening test is reactive, the results should be confirmed by means of a second treponemal test
3493 based on a different analytical method (see Appendix 20).

3494 Testing for HTLV-I antibodies must be performed for donors living in high-prevalence areas.
3495 Likewise, testing is necessary if the donor themselves, the donor's parents or the sexual partners
3496 originate from such areas [16, 17]. Reactive screening results need to be confirmed by immunoblot
3497 analysis and/or specific NAT.
3498 In principle, if a child donor is 18 months old or younger, or has been breastfed in the 12 months
3499 before death, the birth mother should be evaluated for risks associated with HIV, HBV, HCV and HTLV.
3500 In the case of a neonatal donor (i.e. age ≤ 1 month), the required tests should be carried out using both,
3501 a blood sample from the donor's birth mother and one from the newborn. IgG antibodies in the newborn
3502 blood sample are likely to be maternal; therefore, testing for anti-HIV, anti-HCV and anti-HBc is not
3503 suggested in the newborn. However, if a maternal infection is suspected, additional testing for HBs
3504 antigen and HIV-1 RNA, HBV-DNA and HCV-RNA in the newborn sample makes sense, to exclude an
3505 congenital or postnatal infection. In the very rare case of a treated HIV infection in the mother, testing
3506 for presence of HIV-1 in the newborn must be carried out on viral cDNA.
3507 In addition, special screening considerations are applicable to other paediatric donors and
3508 additional testing for communicable diseases (e.g. Zika virus infection, malaria or Chagas disease) may
3509 be indicated. Special attention needs to be paid to putative infections while maternal antibodies may still
3510 be detectable (up to the first 18 months of life).

3511 **5.5.2. Additional tests**

3512 It is well recognised that NAT assays for HIV, HBV and HCV reduce the risk of inadvertent
3513 disease transmission due to the substantial decrease in window period when compared with routine
3514 serological tests [13, 14].
3515 Because NAT assays are more sensitive, serious consideration should be given to also carrying
3516 out NAT tests for HIV, HBV and HCV. Considerations that support the use of NAT assays for each donor
3517 screening include the following:

3518 • The medical and behavioural history obtained from a proxy for a deceased donor can be less
3519 reliable than collecting this information from a living donor.
3520 • There is a risk that a recent exposure to HIV, HBV and HCV (several days prior to death) might
3521 not be detected by serological (antibody) assays due to insufficient amount of antibodies against
3522 the specific virus.
3523 • If the donation includes multiple tissue types and it results in a large number of tissue grafts to be
3524 made available for many recipients, the potential risk is increased if the viral tests selected cannot
3525 detect early infection in a donor.
3526 • The molecular methods used for the screening have to meet the requirements of each individual
3527 pathogen. Haemodilution (see §5.3.2) especially has to be scoped. In case of acute or untreated
3528 chronic infections, viral loads are usually in a range where haemodilution may not cause false-
3529 negative results. But, if low level viraemia is expected, for instance in occult HBV infections, the
3530 PCR method must be as sensitive as possible (currently ≤ 12 IU/mL). Tissue from a donor with
3531 suspected occult HBV infection and haemodilution might not be considered to be safe.
3532 • Molecular assays from deceased donors should be performed in individual samples (see current
3533 legislation of each country), not in pooled samples. Some of these NAT assays are combination
3534 tests that can detect HIV, HCV, and HBV from a single blood specimen in one run, thus improving
3535 the feasibility of routine NAT in donor screening. (In the case of samples from living donors, the

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pooling could be accepted if the national requirements for the comparable NAT testing of blood donors are fulfilled.)

Any relation to high-prevalence areas for specific infections/diseases must be considered carefully. Scientific evidence for risk factors for certain diseases are provided by the European Centre for Disease Prevention and Control [16, 17]. ECDC regularly publishes risk assessments and maps that can be helpful, notably for emerging diseases.

Additional testing that may be considered (depending on the donor's history and/or the characteristics of the tissues or cells donated) includes:

- ABO (AB0) group;
- RhD (D antigen);
- human leucocyte antigen (HLA);
- antibodies to *Cytomegalovirus*, Epstein–Barr virus and *Toxoplasma gondii* might be relevant for donor-recipient risk stratification;
- Hepatitis E virus RNA (i.e. NAT).

Depending on factors like individual travel history and specific current or past clinical abnormalities of the donor as well as the epidemiological situation, the decision can be made to carry out other optional tests, which can include screening for tropical infections such as malaria, trypanosomiasis, viral infections with West Nile virus, Zika virus, etc. The need to perform such assays, or others, must be examined on a case-by-case basis. In the case of paediatric donors, such infections must be reviewed for their impact for mother-to-child transmission.

Results of blood cultures can be very useful tools to aid in the determination of bacteraemia in a donor of tissues and/or cells (see Chapter 10).

5.5.3. Re-tests of samples from living donors (allogeneic use)

Repeat sampling and serology testing is required after 180 days, unless any of the following specific exemption criteria are met:

- if samples from a living donor undergo serology testing and are also tested by molecular tests (i.e. NAT) for HIV, HBV and HCV, which is recommended – because NAT can increase sensitivity in the detection of recently acquired infections, molecular testing of all donors using this technology is highly recommended as standard practice;
- if the tissue/cells come from a living donor and have been processed using an inactivation step that has been validated for the virus(es) concerned;
- if the tissue/cells come from a living donor and will not be stored longer than 180 days prior to use.

Test-kit assays for infectious-disease markers are typically optimised for testing a sample from a living donor. For living donors, initial infectious-disease testing is carried out at the time of donation or, when this is not possible, within 7 days of the donation. In the case of bone marrow and peripheral blood stem-cell collection, blood samples must be drawn for testing < 30 days before donation. Minimum testing requirements are the same as for deceased donors, but there are additional considerations because the donor is available for more testing. For example, more tests could be indicated because there may be unique risks of infectious disease pertinent to a profoundly immuno-suppressed recipient of bone marrow or of similar types of haematopoietic allograft [18].

For testing individuals involved in medically assisted reproduction (MAR), see Chapter 27.

5.5.4. Testing of autologous samples

For autologous donors, if the removed tissues or cells are stored or cultured, they must undergo the same serological tests as for allogeneic donors before they can be transplanted back into the donor. If an autologous donor's blood sample has not been appropriately tested or if a test is indicative for a

relevant infectious disease, this will not necessarily prevent the tissues or cells, or any product derived from them, from being stored, processed and re-implanted in the autologous donor; but this is only true if appropriate storage can provide isolation/segregation to ensure there is:

- no risk of cross-contamination with stored allografts;
- no risk of contamination with adventitious agents;
- avoidance of mix-ups due to misidentification (see Chapters 14 and 15).

SOPs based on risk analyses must be in place to define the criteria for acceptance and rejection for contaminated autologous tissues and cells, or if the autologous donor has not been tested for infectious diseases (see Chapter 2).

5.6. Reporting and documentation of test results

Tissues and cells must be held in 'quarantine' until such time as requirements relating to donor testing have been completed. With this in mind, donor infectious-disease testing should be carried out and reported without delay. Reporting methods must be used that link the donor's unique identifier to the test results, while also keeping the donor anonymous to third parties. Data-security measures are required, as well as safeguards against any unauthorised additions, deletions or modifications to donor test results. There must be no disclosure of infectious-disease test results.

Arrangements between the testing laboratory and the tissue establishment, or the clinical team responsible for use of the donated tissues or cells, should include agreed methods for the reporting of test results to ensure mix-ups are avoided and prevent misinformation. Laboratories and tissue establishments must have policies relating to the management of test results from a donor that may be pertinent to family members and other contacts of the donor or that have implications for public health.

Reporting procedures should ensure that accurate, rapid and verifiable results are provided. In addition, there must be a system in place to ensure prompt alerts using an immediate notification system when an indicative test result for an infectious disease occurs. Other precautionary measures in reporting may include [19] the following:

- where manual systems are still used (although they are not recommended), analysis reports should be cross-checked to ensure that the transcription of test results has been confirmed by two independent assessors (the "four eyes principle");
- using computerised procedures for the transfer of test results from laboratory equipment to the laboratory data-processing management system (e.g. medical records) to eliminate the need for manual transcription of data or oral information;
- using clearly interpretable, computerised graphic symbols to highlight pathologic results;
- recording (semi)quantitative value (e.g. titre, IU/mL) of antibodies and/or the related positivity threshold next to the viral negative/positive result;
- recording the number of copies/mL (or, preferably, IU/mL) of nucleic acid measurement and the limit of detection, as well as (for qPCR systems) the linear range of the assay (if qualitative PCRs are used, then semiquantitative values should be recorded, e.g. Ct-Values, Cp-Values);
- using formal laboratory reporting structures and accreditation or certification pathways to improve quality standards;
- using widely recognised international units of measurement;
- mentioning systematically the name of the kit used (or making available on demand the data of the diagnostic assays, e.g. name, manufacturer).

5.7. Archived samples

All serum and/or plasma samples from the donor that remain after testing have to be aliquoted and stored under appropriate conditions for an appropriate time. If there are no national requirements or risk-analysis-based decisions, a minimum storage time for the archived samples of 1 year after the

3628 distribution of the last tissue from the donor is recommended. In some countries, archiving donor
3629 samples may be required.
3630 Archived samples may be used for several purposes: look-back testing involving a new infectious
3631 agent, development of more accurate or new tests, or if investigating a report of a serious adverse
3632 reaction in a recipient of tissues or cells. A documented risk assessment, approved by the tissue
3633 establishment's RP, should be carried out to determine the fate of all stored tissues and cells following
3634 the introduction of any new donor test that could reasonably be considered to affect safety or quality
3635 (see Chapter 2).

3636
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3681 **Related documents:**
3682 **Appendix 18. Sample haemodilution algorithm**
3683 **Appendix 19. Validation of screening for infectious-disease assays for use with blood from deceased donors.**
3684 **Appendix 20: Treponema pallidum testing**
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3686 # Chapter 6. Procurement

3687 ## 6.1. Introduction

3688 To ensure high standards of quality and safety during the procurement process for tissues and cells, it is
3689 recommended that a quality system be in place in the procurement organisation or the tissue
3690 establishment undertaking the process. This quality system must guarantee adequate training of all
3691 personnel involved, as well as written standard operating procedures (SOPs) that require documentation
3692 for all stages of the process. Procurement professionals should take measures to ensure appropriate
3693 safety and quality parameters are in place.
3694 Procurement of human tissues or cells can take place only after donor consent/authorisation
3695 requirements have been satisfied, as described in Chapter 3. Tissues and cells must also be identified,
3696 packaged and labelled correctly (see Chapter 14) and then transported to the tissue establishment or
3697 clinical team for direct use, in accordance with established requirements.
3698 Chapter 2 sets out the general quality-management expectations about the personnel, facilities,
3699 equipment, materials, procedures and documentation that should be applied when considering the
3700 quality and safety of tissues and cells for human application.
3701 The definition of requirements related to procurement activities requires the acknowledgement
3702 that each of the various types of donation (e.g. deceased donation, either single tissue or multi-tissue,
3703 and living donation) represents a complex of processes and different risk factors that must be considered
3704 in order to assure the quality of the tissues and cells procured as well as the safety of (living) donors and
3705 recipients.
3706 The criteria, including the location and standard of the premises, that apply in procuring tissues
3707 or cells from living donors are equivalent to those for the treatment of patients. However, in deceased
3708 donation there are additional considerations to take into account; as specified in Chapter 3, procurement
3709 from a deceased donor may take place not only in a hospital but in a mortuary or forensic department,
3710 and in those cases it is important to define conditions and requirements to guarantee the quality and
3711 safety of the procured tissues and cells.[1]
3712 Deceased donation of tissues or cells can also occur after organ donation, and in those cases
3713 sterility needs to be ensured throughout the whole procedure, including during organ procurement. If
3714 more than one tissue is to be procured from a single deceased donor, procurement may be performed by
3715 a multi-tissue team or by different tissue-specific teams.
3716 This chapter provides guidance for tissue procurement in general, but with a specific focus on
3717 deceased donors and multi-tissue procurement.

3718 ## 6.2. Personnel

3719 Procurement activities must be undertaken by personnel with appropriate qualifications, training,
3720 expertise and experience. This includes successful completion of a comprehensive technical and/or
3721 clinical training programme, including the broader ethical, legal and regulatory context of procurement.
3722 The training programme must be tailored to the specific tissues or cell types to be procured and will also
3723 depend on whether the procurement is from living or deceased donors.
3724 Persons undertaking procurement must be made aware of the risks and potential consequences if
3725 policies and procedures on procurement are not followed as directed in written SOPs and according to
3726 relevant legislation.
3727 To promote compliance with donor-selection criteria and procurement procedures, the tissue
3728 establishment must have written agreements with each person, clinical team or third-party procurement
3729 organisation involved in carrying out procurement, as well as those collecting critical information used

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in donor selection. The written agreements must include detailed descriptions of expectations and responsibilities related to quality and safety measures, as well as any additional regulatory requirements. A written agreement is not necessary for individuals employed by the tissue establishment responsible for these steps but expectations and responsibilities pertaining to procurement must appear in their job description (see Chapter 2).

6.3. Facilities, equipment and materials

6.3.1. Facilities

Procurement activities must be authorised by the appropriate and competent Health Authority. Each procurement event must take place in an appropriate facility and follow the required clinical/technical procedures (see §6.4). The procedure must take into account the risk of microbial or other contamination of procured tissues and cells, and steps should be taken to minimise the risk. For reasons of privacy and control of contamination, access to the area where procurement takes place must be restricted during the actual procurement of tissues or cells. In addition, the donation of tissues or cells by living donors must take place in an environment that ensures their health, safety and privacy. A risk assessment to determine the suitability of the procurement site, depending on the types of tissues or cells to be procured, must be carried out.

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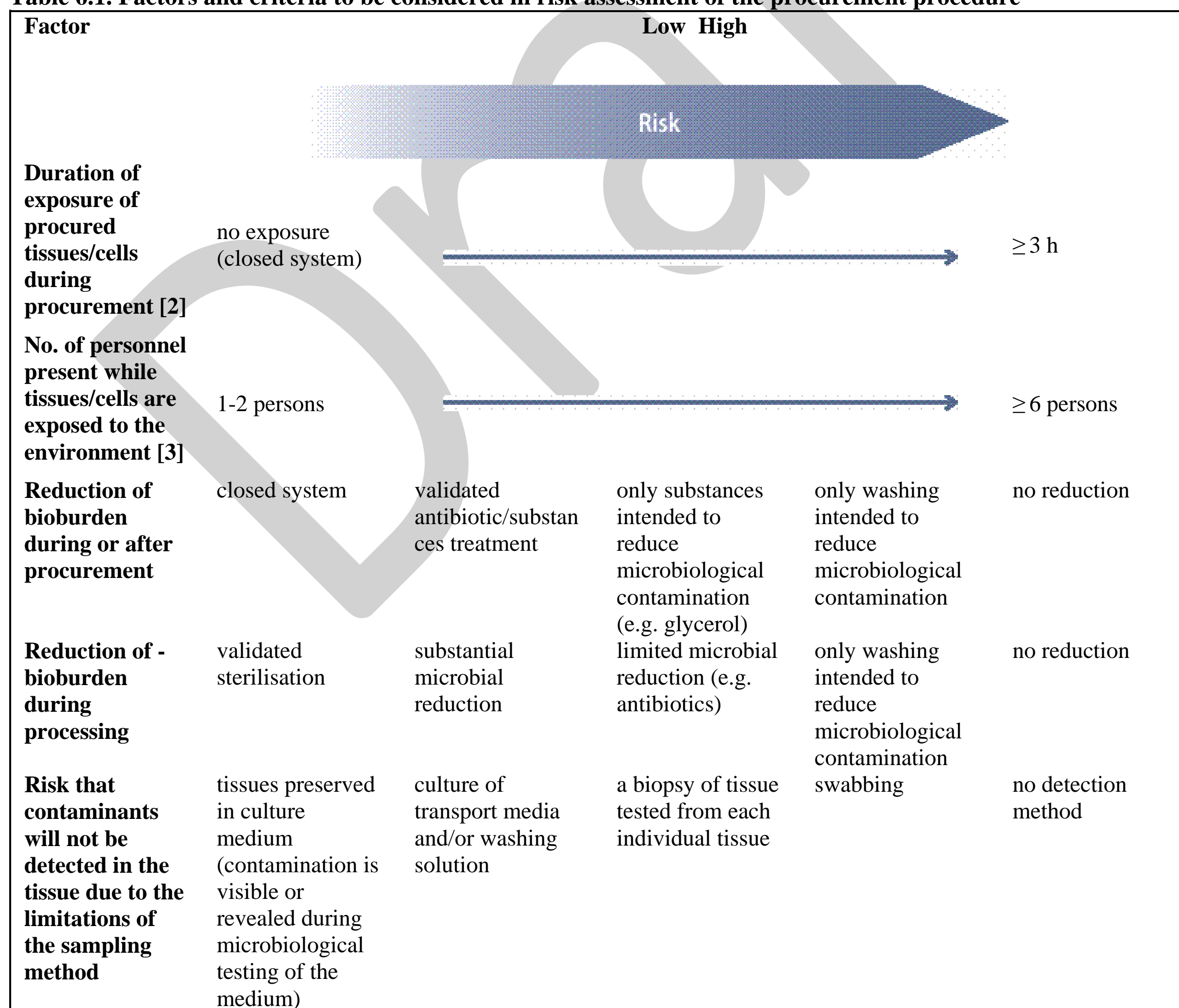
Table 6.1. Factors and criteria to be considered in risk assessment of the procurement procedure



Factor	Low High				
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)				≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1-2 persons				≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of - bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method

Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well vascularised site	large durable implant in a well vascularised site	direct application into the blood stream (infusion)

3748
3749 It is highly recommended that the facility where procurement takes place is:
3750 a. of adequate size in the floor space, work-tops and benches that will be used;
3751 b. appropriately located to ensure cleanliness and privacy;
3752 c. furnished with sufficient and suitable lighting;
3753 d. in a good state of repair;
3754 e. free of pests; and
3755 f. able to provide a sufficiently clean or cleanable environment that will not increase the risk of
3756 contamination of the cells or tissues during their procurement.

3757 Before procurement, steps to minimise the potential for contamination must include cleaning of
3758 all work surfaces with an appropriate and effective disinfectant. The procurement area must also be
3759 cleaned appropriately after the procurement, including proper and safe disposal of single-use
3760 instruments, consumables and any other waste, including clinical waste that poses a biohazard. Any re-
3761 usable instruments will need to be cleaned and sterilised. If a tissue establishment (or third party carrying
3762 out the retrieval) uses the general services of the host facility to clean the procurement area and/or
3763 sterilise any re-usable instruments, the tissue establishment must have a written agreement with the host
3764 facility and the procedures used must be inspected and validated.

3765 *6.3.1.1. Defining the requirements of a procurement area*
3766 Procurement of tissues and cells may take place in various facilities, ranging from a hospital
3767 operating room, tissue establishment, hospital clinic, mortuary, funeral home or care home, to a donor's
3768 own home. These facilities can be broadly categorised as:
3769 • operating theatre or equivalent;
3770 • dedicated procurement area with routine air-quality monitoring and controlled cleaning (e.g.
3771 tissue establishment procurement room);
3772 • dedicated clean area (controlled cleaning);
3773 • non-dedicated area, with local cleaning of the procurement space.

3774 A risk assessment based on the factors detailed in Table 6.1 will help define an appropriate
3775 procurement area, including air quality, depending on the level of risk and any subsequent steps taken
3776 during processing. Taking into account the criteria defined in Table 6.1, a risk assessment might reach
3777 similar conclusions to the cases exemplified in Tables 6.2 to 6.7. In each case, the yellow background
3778 shows the typical options in practice.
3779

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3780

3781 **Table 6.2. Example of musculoskeletal tissue recovery procedure with the specified characteristics**

Factor	Low	Risk			High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤1h	1-2h	2-3h	≥3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 persons	2-3 persons	4 persons	5 persons	≥6 persons
Reduction of bio-burden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	washing intended to reduce microbiological contamination	no reduction
Reduction of -bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues or cells preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable clinical application in a well-vascularised site	large durable clinical application in a well-vascularised site	direct application into the blood stream (infusion)

3782

3783 *6.3.1.2. Example of musculoskeletal tissue recovery procedure with the specified characteristics*

3784 • Tissues are exposed to the environment for a long period of time (medium/high risk)
3785 • The procedure is performed by two recovery members, plus one nurse responsible for tissue
3786 packaging and one circulating assistant (medium risk)
3787 • During procurement, tissues are washed with sterile water to reduce surface microbiological
3788 contamination (medium/high risk)
3789 • During processing an antibiotic decontamination will be applied to musculoskeletal tissues
3790 (medium risk)
3791 • During procurement and processing the sampling method used is swabbing of each tissue so
3792 detection of contaminants might be missed (medium/high risk)
3793 • Musculoskeletal tissues are usually used as durable grafts and implanted directly into a well-
3794 vascularised bed in the recipient (medium/high risk)

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Probable risk assessment: It is considered a medium- to high-risk procedure and for this reason the conclusion of the risk assessment is likely to be that musculoskeletal tissues should be procured in an operating theatre environment or equivalent [4].

Table 6.3. Example of sclerocorneal button recovery procedure with the specified characteristics

Factor	Low	Risk			High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤1h	1-2h	2-3h	≥3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 person	2-3 persons	4 persons	5 persons	≥6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the blood stream (infusion)

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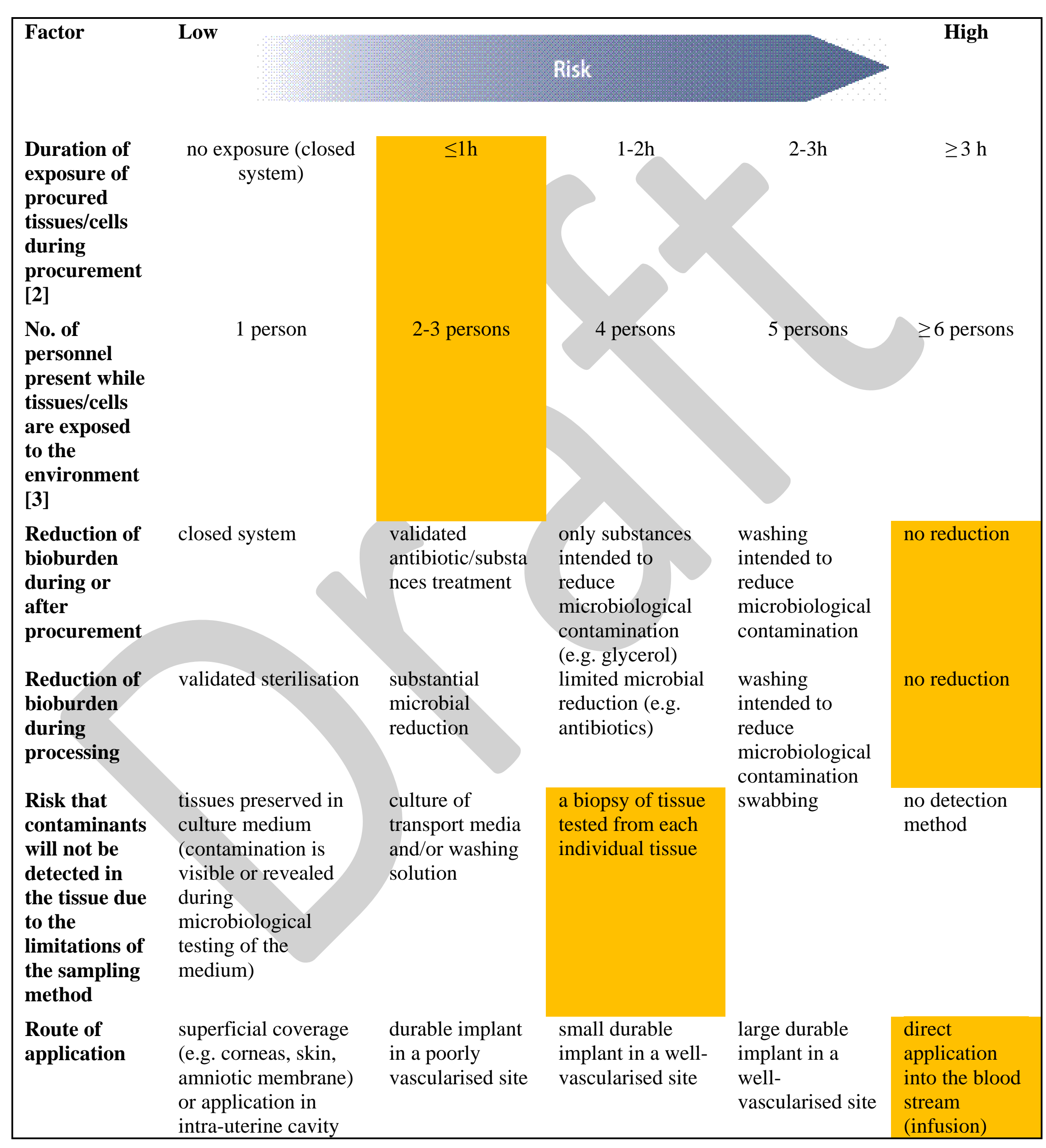
6.3.1.3. Example of sclerocorneal button recovery procedure with the specified characteristics

- Tissues are exposed to the environment for a short period of time (low/medium risk)
- The procedure is performed by one recovery member (low risk)
- Before procurement, ocular surface is cleaned with a validated combination of iodine and antibiotic solution to reduce surface microbiological contamination (low/medium risk)
- No reduction of bioburden is applied during corneal processing/evaluation (high risk)
- After procurement, corneas are preserved into a culture medium allowing the evaluation of possible contamination (low risk)
- Cornea grafts are used as superficial coverage (low risk)

Probable risk assessment: It is considered a low- or medium-risk procedure and for this reason the conclusion of the risk assessment is likely to be that it is not considered necessary to procure eyes or corneas by *in situ* excision in a location with controlled, defined air quality. However, steps must be

taken to reduce the bioburden on the ocular surface before procurement, especially for corneas procured by *in situ* excision, and a local sterile field must be created around the eye. If the whole eye is procured, further steps must be taken in the eye bank to reduce bioburden before excision of the corneoscleral disc. Furthermore, for corneas stored by organ culture, microbiological testing of the organ culture medium during corneal storage is essential to further mitigate the risk of microbiological contamination.

Table 6.4. Example of bone-marrow recovery procedure with the specified characteristics



Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤1h	1-2h	2-3h	≥3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 person	2-3 persons	4 persons	5 persons	≥6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the blood stream (infusion)

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6.3.1.4. Example of bone-marrow recovery procedure with the specified characteristics

- Tissues are exposed to the environment for a short period of time during aspiration procedure (low/medium risk)
- The procedure is performed by a low number of recovery members, usually two (low/medium risk)
- No bioburden reduction is performed during recovery (5 points) or processing (high risk)

- During procurement, a biopsy of bone-marrow is obtained for microbiological control (medium risk)
- Bone-marrow graft is used in immuno-suppressed patients and it is injected directly into the blood stream (high risk)

Probable risk assessment: It is considered a medium- to high-risk procedure and for this reason the conclusion of the risk assessment is that procurement needs to be performed in an operating theatre or similar.

Table 6.5. Example of peripheral blood stem-cell recovery procedure with the specified characteristics

Factor	**Low**				**High**
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤1h	1-2h	2-3h	≥3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 person	2-3 persons	4 persons	5 persons	≥6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the blood stream (infusion)

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6.3.1.5. Example of peripheral blood stem-cell recovery procedure with the specified characteristics

- Peripheral blood stem cells are collected and processed in closed systems without exposure to environment (low risk)
- The procedure is performed by one person (low risk)
- Bioburden reduction during recovery is not needed because of the use of a closed system (low risk)
- No reduction of bioburden is performed (high risk)
- At the end of collection/before processing, and at the end of processing, a blood sample is obtained for microbiological control (medium risk)

3847 • Peripheral blood stem cells are injected directly into the blood stream (high risk)

3848 **Probable risk assessment:** It is considered a low- or medium-risk procedure, mainly because of
3849 the use of closed systems during procurement and processing. Therefore, particular environmental
3850 requirements may not be necessary and the peripheral blood stem cells may be collected in a blood-
3851 donor clinic.

3852 **Table 6.6. Example of female gametes recovery through transvaginal procedure with the specified**
3853 **characteristics**
3854

Factor	Low	Risk →			High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤1h	1-2h	2-3h	≥3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 person	2-3 persons	4 persons	5 persons	≥6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the blood stream (infusion)

3855

3856 *6.3.1.6. Example of female gametes recovery through transvaginal procedure with the specified*
3857 *characteristics*

3858 • Female gametes are collected in an open system during a procurement procedure that mostly
3859 takes on average 20-30 minutes (low risk)
3860 • The collection procedure is usually performed by one person (medical doctor), and there is
3861 assistance from a second person (nurse or midwife) (low risk)
3862 • After collection, the oocytes are processed in an open system; they are collected from the
3863 follicular fluid and put in a small dish containing washing media supplemented with antibiotics;
3864 after this step, oocytes are individually selected and put in culture droplets containing media
3865 supplemented with antibiotics; these droplets are covered with oil (low risk)

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3866 • During processing, no sampling for microbiological control is usually done; according to Table
3867 6.6, this would result in a high risk, but retrospective validation of this consolidated approach
3868 for the collection and subsequent processing of oocytes shows that there is a very low risk of
3869 microbial contamination
3870 • Female gametes are subsequently used for fertilisation and the resulting embryos are considered
3871 a low-risk application because they are clinically applied in the intra-uterine cavity (low risk)

3872 **Probable risk assessment:** It is considered a low-risk procedure, the risk being mainly reduced
3873 by the use of substances for bioburden reduction during procurement and processing, as well as by its
3874 clinical application. However, considerations of the donor's situation during the collection procedure
3875 (under the effect of sedatives) may recommend that procurement is performed in an operating theatre
3876 (where particular environmental requirements may be necessary). Additional specific situations in MAR
3877 are described in Chapter 27.

3878 **Table 6.7. Example of skin recovery procedure with the specified characteristics (glycerol preservation)**
3879

Factor	**Low**	Risk →			**High**
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤1h	1-2h	2-3h	≥3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 person	2-3 persons	4 persons	5 persons	≥6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the blood stream (infusion)

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6.3.1.7. Example of skin recovery procedure with the specified characteristics (glycerol preservation)

- Tissues are exposed to the environment for a short period of time: less than 1 hour (low/medium risk)
- The procedure is performed by two recovery members and one circulating assistant (low/medium risk)
- After procurement, tissues are placed into a container with glycerol that is intended to reduce the microbiological contamination (medium risk)
- During processing, a change of preservation media to higher glycerol concentrations is performed, obtaining a substantial microbial reduction (low/medium risk)
- During procurement, a culture of transport media is performed as sampling method to detect microbiological contamination (low/medium risk)
- Glycerolised skin grafts are used as superficial coverage into the recipient (low risk)

Probable risk assessment: It is considered a low- or medium-risk procedure and for this reason the conclusion of the risk assessment is likely to be that glycerolised skin grafts could be procured in a non-dedicated area with local cleaning, although procurement in more controlled areas will decrease the risk of contamination during procurement. Differences can apply if other preservation methods, like cryopreservation, are used and then a new risk assessment should be done.

6.3.2. Equipment and materials

Materials (i.e. consumables and reagents) and equipment (i.e. surgical instruments, packaging and containers) used during procurement must be managed in accordance with standards and specifications and with due regard for relevant national and international regulations, standards and guidelines for the intended use of the donated tissues and cells (see Chapter 2). Validated sterile instruments, CE (Conformité Européenne)-marked devices (where available) and sterile single-use materials (e.g. drapes, gloves, fluids) must be used for tissue and cell procurement. Instruments or devices must be of good quality, validated or certified specifically (e.g. surgical grade) for procurement, and must be maintained in good working order. This must include visual inspection and scheduled calibration of devices, where appropriate, against relevant defined standards at specified intervals. Routine maintenance inspections (validation procedures), at least annually, of equipment used for procurement are encouraged and a re-validation assessment is required whenever repairs or modifications have occurred. Procurement personnel must receive appropriate training, supported by records, on the proper use of equipment.

Where possible, use of single-use instruments for procurement is recommended. When re-usable instruments are used, a validated cleaning, disinfection, packaging and sterilisation process for removal of infectious agents must be used and each event documented. A system must be in place that allows traceability and tracking of critical equipment and materials to each tissue- or cell-procurement event and to the donor.

In EU member states, critical reagents, materials reagents and materials must meet documented requirements and specifications, and when applicable, the requirements of Directive 93/42/EEC concerning medical devices and Directive 98/79/EC on *in vitro* diagnostic medical devices.

Personnel conducting procurement activities must be provided with protective clothing appropriate for the type of procurement. Usually, this will extend to being scrubbed as for surgery and involve wearing a sterile gown, sterile gloves, glasses and a face shield or protective mask. Approved materials necessary for reconstruction of a deceased donor's body must be provided to allow this step to be completed effectively.

6.3.2.1. Identification, packaging, containers and labelling

At the time of procurement, tissues and cells must be uniquely identified. They must be packaged so as to minimise the risk of environmental contamination. Labelling must be witnessed and the labels must be appropriate to ensure identification and traceability of tissues and cells. Labels must be resistant to storage conditions to avoid the loss of identification of tissues and cells.

3930 Guidance on coding, packaging and labelling is provided in Chapter 14.

3931 6.4. Procedures

3932 Written SOPs for procurement must be in place, based on the requirements of the relevant Health
3933 Authority, the recommendations laid out in this Guide and the expectations of the tissue establishment
3934 or end-user needs. These SOPs must outline the correct steps to be taken for each stage of procurement.
3935 Procedures that ensure contamination control must be applied, including use of aseptic techniques,
3936 sterile materials and equipment and appropriate clothing for the personnel conducting the procurement
3937 (see §6.2 and §6.3.2). Review of procurement SOPs by a Responsible Person must be undertaken at least
3938 annually (or as required), and updates may be necessary owing to clinical, scientific or technical
3939 progress. Procedures must be authorised and appropriate for the type of donor and the type of tissue or
3940 cells procured, and must be standardised [5].

3941 The SOPs must be readily accessible so that procurement personnel can follow the required steps,
3942 including:

3943 a. verification of the donor's identity and what constitutes evidence of donor (or the donor family's)
3944 consent or authorisation (see Chapter 3);
3945 b. assignment and appropriate use of a unique identifier/code (see Chapter 14);
3946 c. knowledge of selection (risk) criteria required for donor assessment, including physical
3947 examination of the donor (see Chapter 4);
3948 d. knowledge of the types of blood and other samples required for mandatory laboratory tests to
3949 ensure that they are of appropriate quality(see Chapter 5);
3950 e. steps that minimise the risk of microbiological contamination during procurement (see this
3951 chapter, as well as Chapters 2, 10 and 17-32);
3952 f. procurement steps that protect the properties of the tissue and cells required for clinical use (see
3953 this chapter and Chapters 17-32);
3954 g. for deceased donation, how to reconstruct the donor's body so it is as similar as possible to its
3955 original anatomical appearance;
3956 h. considerations for packaging, labelling and transportation of procured tissues or cells to the tissue
3957 establishment or, in the case of direct distribution, to the clinical team responsible for their human
3958 application or direct use (see this chapter and Chapters 12 and 14);
3959 i. considerations for collecting, packaging, labelling and transporting samples of donor blood or
3960 other samples to the laboratory for testing (see this chapter, and Chapters 5 and 14);
3961 j. procedures that protect the health and safety of the living donor (see Chapters 18 and 24-32).

3962 In addition, the tissue establishment is expected to have procedures in place to notify, without
3963 delay (see Chapter 16), other tissue establishments or the relevant Health Authority of all available
3964 information about:

3965 a. knowledge of deviations from approved procedures that have occurred or that are suspected to
3966 have occurred; and/or
3967 b. any serious adverse reaction in a living donor that may influence the quality and safety of the
3968 tissues or cells procured.

3969 To minimise the risk of tissue or cell contamination by procurement personnel who may be
3970 infected with a transmissible disease, policies and procedures must be established and followed to
3971 address this risk.

3972 Additional procedures and policies that minimise the risk of microbiological contamination
3973 during procurement must be considered (see also Chapters 17-32, including those listed here):

3974 a. the maximum number of personnel permitted to be present during procurement must be defined
3975 and respected;

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3976 b. preparation of the donor's skin must follow the recommended standards of practice used for
3977 surgical patients and must occur at the beginning of procurement using an appropriate
3978 antimicrobial agent designed for this purpose;
3979 c. the procedure for skin disinfection should account for the elimination of bacterial spores as well
3980 as vegetative micro-organisms and it should therefore include suitable disinfectants, their
3981 concentrations and durations of exposure;
3982 d. before use, all materials and equipment must be visually inspected by procurement personnel to
3983 ensure that they meet specifications (e.g. sterile, seals not broken, equipment functioning as
3984 expected);
3985 e. for deceased donation, it is advisable to procure tissue before the autopsy takes place but, if this
3986 is not possible, detailed procedures must be written to address the increased potential for
3987 contamination when procurement takes place after autopsy.

3988 Procurement must include procedures that protect those properties of tissues and cells required
3989 for their ultimate clinical use. These are described more fully in Part B of this Guide (the tissue-specific
3990 chapters), but generally include:
3991 a. *post mortem* procurement time limits – it is recommended that tissue should be procured within
3992 24 h after death if the body has been cooled or refrigerated (with the aim of reducing
3993 microbiological growth) in the first 6 h after death, or within 12 h of death if the body has not
3994 been refrigerated; alternative time limits for procurement should be validated by quality
3995 assessments and tests for microbiological contamination; it may be possible to extend
3996 procurement times up to 48 h after death if processing has been validated to guarantee quality
3997 and microbiological safety, in which case the blood samples for serological testing should still
3998 be taken within 24 h after death to minimise the risk of haemolysis (see Chapter 5 for details on
3999 sample collection);
4000 b. preservation of important anatomical structures and other tissue or cell characteristics;
4001 c. temperature requirements during storage and transport to the next destination;
4002 d. avoidance of delays in transport due to time limits in place for processing after procurement.

4003 Instead of specialised procurement teams recovering different tissues from a deceased donor, a
4004 multi-tissue procurement team consists of a group of individuals who are trained to procure all tissues
4005 for which there is consent. The roles of the individual multi-tissue team members must be defined by
4006 SOPs.
4007 The main advantages of a multi-tissue procurement model are:
4008 a. better co-ordination, because all tissues are procured by the same team;
4009 b. less time taken to procure all tissues, thereby decreasing the risk of microbial contamination because
4010 of long warm ischaemia times;
4011 c. fewer equipment and consumables resources needed (e.g. same draping may be used to procure
4012 cardiovascular and musculoskeletal tissues).

4013 In every deceased-donor procurement team, an appropriately trained senior person must take
4014 overall responsibility to ensure that SOPs are adhered to and that the following tasks are carried out to
4015 the required standards:
4016 a. identification of the donor (Appendix 22);
4017 b. review of donor documentation, including medical history, laboratory tests (if completed),
4018 lawful consent/authorisation;
4019 c. physical examination of the donor (Appendix 15);
4020 d. organisation and co-ordination of the procurement;
4021 e. evaluation of abnormal procurement findings;
4022 f. review of tissue packaging and labelling;
4023 g. review of donor reconstruction;

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h. completion of all required procurement documentation.

The sequence in which the various tissues are procured must be well defined to assure the quality of each type of tissue. The recommended procurement sequence, whether carried out by separate teams or by a multi-tissue team, is: skin, eyes/corneas, cardiovascular and musculoskeletal. Justification for this recommended procurement flow includes the following reasons:

- Skin is the first procured tissue because the donor is placed in a prone position to obtain skin from back and lower limbs, and the support provided by the presence of musculoskeletal tissues (in particular, bones) facilitates the procedure.
- Eyes are recommended to be procured after skin to avoid eye bleeding from the sockets if the donor has to be placed in a prone position following enucleation of the eyes.
- Cardiovascular and musculoskeletal tissues are recommended to be procured last because the same donor draping may be used. Some cardiovascular tissues (e.g. femoral arteries) may be procured simultaneously with the musculoskeletal tissues.

Where a tissue donor has already donated organs, all surgical approaches to obtain the organs must have been sutured to maintain as far as possible the sterility of thoracic and abdominal tissues before their procurement. If the procurement is performed simultaneously with organ procurement, the sequence varies: starting with the tissues from the cavities open for organ recovery, thorax and abdomen (arteries, heart for heart valves or vertebral bodies), then the recommended sequence of skin, eyes, cardiovascular and musculoskeletal should be followed. It is important that all the procurement teams involved know that tissues will be procured after organs, first to prepare the body before starting surgery, and second to guarantee sterile conditions during the whole procedure and to minimise the risk of cross-contamination.

Efforts should be made to ensure that procurement procedures do not unnecessarily interfere with funeral arrangements or other formalities such as religious or cultural rituals. If this is not possible, the donor's family must be informed at the time of consent. Timely and effective communication with all parties involved can help to meet expectations in regard to delays, as well as aesthetic considerations when tissues are procured from areas of the body that may be visible (e.g. if the body is to be viewed subsequently by the family and those attending the funeral).

6.4.1. Processing at the procurement stage

Microbiological safety during the procurement of tissues or cells must always be considered; but control of contamination and cross-contamination at the procurement site is typically less stringent than the controls applied in a tissue establishment (see Chapters 2, 10 and 17-32). Therefore, simultaneous undertaking of processing steps during the procurement phase, or in the procurement area, is not recommended and is avoidable. However, if processing, including shaping, cleaning, sizing and final packaging (for direct distribution) at the procurement site is unavoidable, its duration and extent should be limited to the minimum necessary, and a Grade A air-quality environment (surrounded by, at least, Grade D air quality) for the processing steps is desirable (e.g. a laminar flow cabinet located in the operating room). Records supporting the validation of the processing site must be available for inspection. If this level of control is not possible, an in-process (active) environmental-monitoring method must be used: preferably, active air monitoring using a viable particle counter and culturing method or, as a minimum control, using microbiological settle plates. Sample cultures of the tissues or cells procured should also be taken (see Chapter 10) and an appropriately validated culture method must be used (see Chapter 2). Ultimately, the procurement environment, if it is also used as a processing environment, must be specified and must achieve the quality and safety required for:

a. the types of tissues and cells procured;
b. the types of processing steps and tissues or cells that will be used (e.g. none; exposure only to antibiotics; a validated inactivation method; or, a validated sterilisation method);

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4071 c. the types of clinical application (as well as consideration of the immune status of the recipient, if
4072 applicable).

4073 Selection of the use of suboptimal conditions must be supported by written justification and be
4074 authorised by the relevant Health Authority.
4075 From the premise that processing at a procurement site is not considered Good Tissue Practice
4076 and all efforts should be directed towards avoiding this practice, Table 6.2 shows recommendations for
4077 best practice when it is unavoidable. Four main factors are taken into account:
4078 a. Procurement air quality: the environment where procurement is done. Two situations are possible:
4079 i. a dedicated area with air-quality monitoring, or an operating theatre,
4080 ii. a dedicated clean area, or a non-dedicated area.
4081 b. Processing air quality: the environment where the processing step is done, following procurement.
4082 Two situations are considered:
4083 iii. Class A (i.e. laminar flow cabinet placed in the procurement area),
4084 iv. non-Class A.
4085 c. Recovering and processing time: duration of the whole process (including procurement and
4086 processing) where tissues are exposed to the procurement and processing environments. Times
4087 over 3 hours are considered as critical.
4088 d. Type of processing: type of process to which tissues are subjected. Various procedures are
4089 considered:
4090 v. validated decontamination method,
4091 vi. terminal sterilisation,
4092 vii. other procedures, such as non-validated decontamination methods, washing …

4093 Some considerations obtained from the algorithm (Figure 6.2) are:
4094 a. When procurement is done in a dedicated clean area (without air-quality control) or a non-
4095 dedicated area, it is recommended to establish in-process environmental monitoring as well as
4096 using microbiological settle plates during the whole process.
4097 b. When processing is not done in a Class A environment, or when the duration of the procurement
4098 and processing procedures is longer than 3 hours, it is recommended to establish in-process
4099 environmental monitoring as well as using microbiological settle plates during whole process.
4100 c. When a validated decontamination method or terminal sterilisation is used, validation records are
4101 required, independently of the conditions of procurement and processing steps.
4102 d. The following situations are considered to be suboptimal conditions, where validation records,
4103 written justification and authorisation from competent authority are required:
4104 i. Procurement in a dedicated clean area (without air-quality control) or a non-dedicated
4105 area, with Processing not Class A;
4106 ii. Procurement in a dedicated clean area (without air-quality control) or non-dedicated
4107 area, with Processing in Class A but non-validated decontamination method or no
4108 reduction used.

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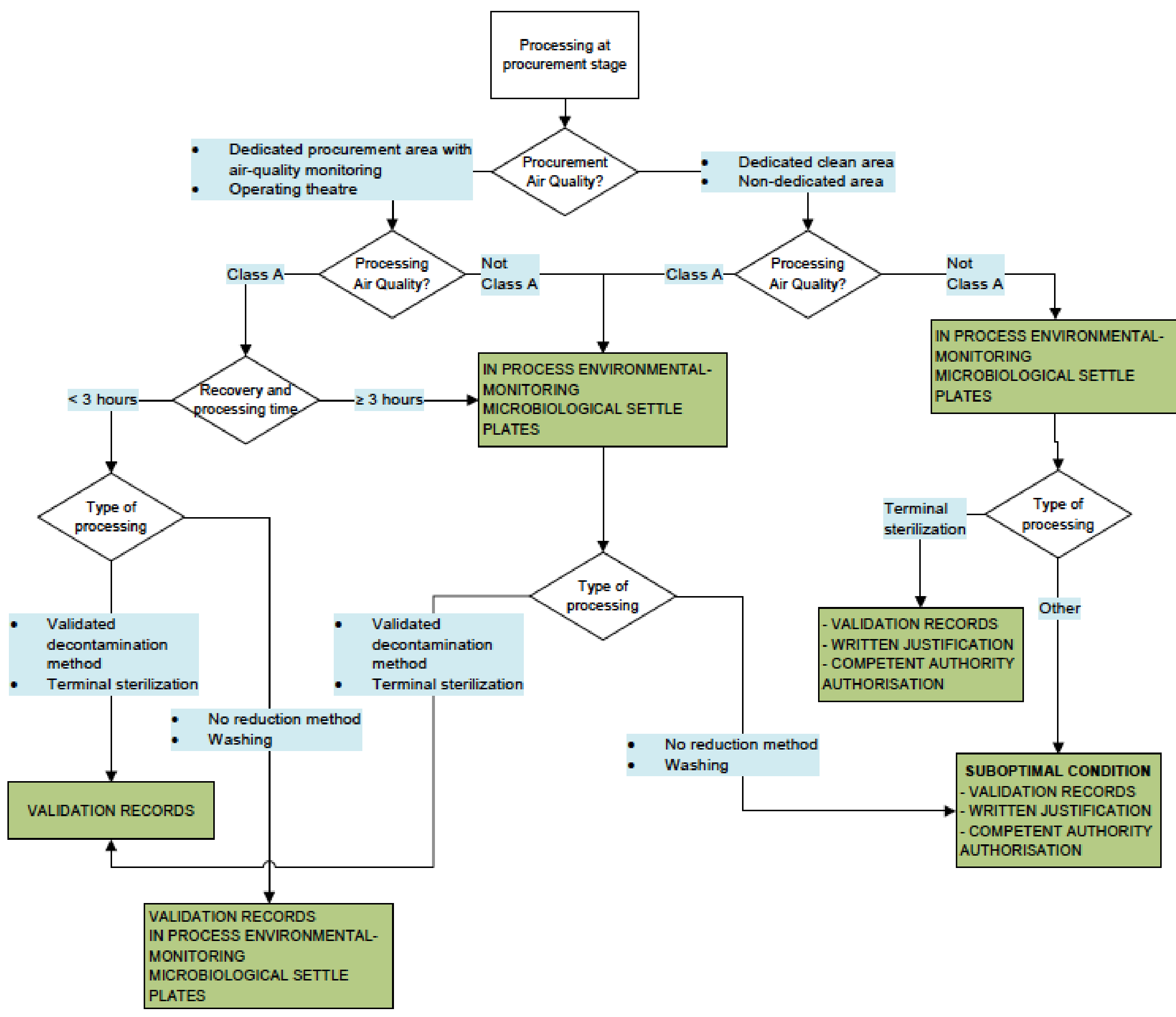

4110 **Figure 6.1. Environment quality: algorithm for processing at procurement stage**

4111 **6.4.2. Temporary storage and transportation to the tissue establishment**

4112 Once the tissue is procured and until it arrives at the tissue establishment, critical variables related
4113 to maintaining the quality of the tissues or cells (e.g. temperature, sterile packaging) must be controlled
4114 (see Chapter 14). Records to demonstrate compliance with specified storage conditions must be
4115 completed and maintained.

4116 6.5. Documentation

4117 Procurement is a critical activity. Therefore tissue establishments must have procedures in place that
4118 address the retention of procurement records, which must include descriptive documentation of the steps
4119 taken, the materials and equipment used, and identification of the personnel involved. Such records must
4120 be clear and legible, protected from unauthorised amendments, retained and readily retrievable
4121 throughout a specified retention period, and must comply with data-protection legislation. Procurement
4122 records must be sufficiently detailed to facilitate robust and reliable traceability, to provide a complete
4123 history of the work undertaken and to be capable of linking the records to the particular donor of the
4124 procured tissues and cells (see Chapter 15). When tissues and cells are to be sent across national borders,
4125 potential language barriers should be addressed and a common language agreed for all documentation
4126 related to donors, tissues and cells.

A unique identifier (e.g. a donation number for a donation event and/or a donor identification number) must be allocated to the donor as well as the procured tissues and cells (see Chapter 14). This coding must be in place to ensure an effective and accurate system capable of tracking tissues throughout all stages, including an identifiable link to the procurement steps. For each donor, there must be a record containing the donor's identity (i.e. given name, family name, date of birth, sex). If a mother and child (both living) are involved in the donation, records must indicate not only the name and date of birth of the mother, but also the name (if determined) and date of birth of the child. These coded data should be entered in a registry maintained for this purpose.

In summary, before the procurement of tissues and cells may proceed, an authorised person (e.g. the team leader in a procurement team) must confirm and record the following as part of the procurement record:

a. donor identification;
b. that consent for the procurement has been obtained in accordance with local laws;
c. how and by whom the donor has been reliably identified.

To ensure that all steps are traceable and verifiable, the tissue establishment (or procurement organisation) must produce a report, recorded at the time of procurement, which must be forwarded without delay to the location where processing takes place. Care should be taken to maintain donor confidentiality if the procurement report is forwarded to the recipient's clinical team. This procurement report, depending on the type of donor, could contain the following:

a. donor identification data (given name, family name, date of birth and sex, as well as how and by whom the donor was identified, or donor identification in the case of an unrelated haematopoietic stem-cell (HPC) donor);
b. unique coding number, which will be either the donation identification sequence of the Single European Code for EU countries, or a code generated by a Health Authority or by use of an internationally recognised coding system such as ISBT 128 or Eurocode (Chapter 14);
c. the environmental conditions of the procurement facility, i.e. location or description of the physical area where procurement took place (see Appendix 21);
d. a list of observations made during the physical examination of the donor's body – but, for a living donor, only when such an examination is justified (see Appendix 15);
e. a description and identification of procured tissues and cells, including samples for testing of infectious diseases;
f. the identification of the person who has overall responsibility for the procurement session (including his/her signature);
g. date, time (where relevant, start and end times) and location of procurement;
h. the type, volume, manufacturer and lot/batch/serial number of reagents, additives and the tissue and cell transport solution(s) used;
i. name and address of the tissue establishment;
j. name and destination of the tissues and cells.

In addition, for procurement of tissues or cells from a deceased donor, this report must contain:

a. a sufficiently detailed summary of the events surrounding death;
b. the date and time of donor death and tissue procurement (and, where relevant, start and end times) to facilitate determination of the time interval from death to procurement;
c. the conditions under which the donor body was kept before procurement (whether or not the donor body was cooled or refrigerated and, where appropriate, the time when cooling or refrigeration began and ceased);
d. if possible, whether procurement took place before or after autopsy and whether or not an autopsy is planned;
e. when applicable, a description of other tissues and cells from the same donor sent to different tissue establishments, including their identification;

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4176 f. if applicable, information regarding reconstruction of the donor's body.

4177 If procurement from a living donor involves a directed donation, the recipient's identification
4178 must be documented to avoid confusion.

4179
6.6. References

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4190 **Related documents:**
4191 **Appendix 21. Sample form to assess the suitability of the working environment;**
4192 **Appendix 22. Sample donor identification form.**
4193

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Chapter 7. Premises

7.1. Introduction

In general, tissue establishments must have suitable facilities to carry out the activities for which accreditation/designation/authorisation or licensing is sought. This chapter provides generic guidance on the facilities used for processing, testing and storing of tissues and cells. Processing of tissue and cells, while exposed to the environment, must take place in an environment with specified air quality and cleanliness in order to minimise the risk of contamination, including cross-contamination between cells and tissues of different donors. This chapter gives guidance on creating, implementing and maintaining a validation master plan (classification and qualification) and monitoring plan in order to gain assurance that the cleanrooms are performing adequately and that the aseptic processing of tissue and cells is monitored (see also Chapter 2). Tissue- and cell-specific guidance on selecting the appropriate air quality for processing is given in part B of this guide.

7.2. Requirements of storage facilities

Tissue establishments should have specific storage facilities/areas for the storage of tissues and cells. Such storage areas should be:

a. designated;
b. located in a secure area, and access must be limited to authorised personnel;
c. of sufficient capacity to allow orderly storage of the various categories of tissues/cells:
   - in quarantine;
   - released for processing;
   - rejected;
   - returned;
   - recalled;
   - for research use;
d. covered by an adequate management system, ensuring clear segregation of each category of tissues and cells. Physical and automated tissue-storage-management systems are both accepted as long as the risks of mix-up between categories and cross-contamination of tissues and cells of different donors are excluded. If any automated management system is used to manage the location of the tissues and cells, documented evidence should be provided to demonstrate the capability of the system to assure safe storage (see Chapter 13);
e. clean and dry, and maintained within an acceptable temperature range. Where special storage conditions are required (e.g. specific temperature and/or humidity) these should be specified, maintained and monitored. The necessary air-conditioning capacity for the storage area must be calculated, based on the actual heat load of the equipment and the environmental factors (see Chapter 9).

In addition, printed packaging and labelling materials may be considered critical and special attention should be paid to their safe and secure storage.

The design of a cryostorage room (e.g. storage rooms equipped with liquid nitrogen tanks or equipment using liquid nitrogen) must comply with applicable regulations and safety requirements of the relevant country. Items related to safety should include at least:

a. good ventilation;
b. oxygen-level monitoring with a local audio and visual alarm;
c. a floor resistant to liquid nitrogen;

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4237 d. adequate space to contain the necessary freezers and tanks, including back-up systems;
4238 e. easy access to all the storage devices, with a smooth pathway to and from the facility for liquid
4239 nitrogen supply and for prompt removal and transfer of tissues and cells in case of emergency;
4240 f. personal protective equipment available for use, which may include items such as cryo-gloves,
4241 safety goggles, cryo-aprons and respirators;
4242 g. a specific SOP already in place to support the safety issues.

4243 A system to monitor all the alarms, including temperature and oxygen level in the room and the
4244 level of liquid nitrogen in the tanks, is essential. Personnel must be trained to react to different alarms.
4245 Personnel need to be trained to use personal protective equipment.

4246 7.3. Requirements of processing facilities

4247 Processing facilities must be designed, classified, qualified and monitored to ensure that the air quality
4248 is appropriate for the processes carried out. International standards, such as the EU Guidelines to good
4249 manufacturing practices for medicinal products for human and veterinary use (known as GMP) [1] and
4250 ISO 14644 guidelines for Cleanrooms and associated controlled environments [2], provide information
4251 to help achieve the appropriate air quality.
4252 Processing of tissues and cells should be carried out in cleanrooms. In these cleanrooms, the
4253 concentration of airborne particles (viable and non-viable) must be controlled to specified levels. Each
4254 processing operation requires an appropriate level of environmental cleanliness in the operational state
4255 to minimise the risks of particulate or microbial contamination. According to GMP, four grades can be
4256 distinguished.

4257 • Grade A: The local zone for high-risk operations provided by localised airflow protection, such
4258 as laminar airflow workstations, isolators or restricted access barrier systems (RABS).
4259 Unidirectional airflow systems should provide a homogeneous air speed in the range 0.36-0.54 m/s
4260 (guidance value) across the whole of the Grade A area (GMP, Annex 1). Maintenance of the
4261 unidirectional airflow should be demonstrated and qualified.

4262 • Grade B: For aseptic operations, this is the background environment for the Grade A zone. Lower
4263 grades can be considered as defined in the tissues- and cells-specific chapters of this guide (Part
4264 B). The risk-assessment tool for defining the air quality can be used to select the background
4265 environment for the Grade A zone (see §7.4).

4266 • Grades C and D: Clean areas for carrying out less critical stages in the processing and storage of
4267 tissues and cells. These cleanliness grades can be considered where isolator technology is used.

4268 Different cleanroom standards are compared in Table 7.1 [1, 2]. Whichever classification is
4269 applied, facilities should have:
4270 a. floors, walls and ceilings of a non-porous material with smooth surfaces to minimise the
4271 shedding or accumulation of viable and non-viable particles and to permit the repeated
4272 application of cleaning agents and disinfectants;
4273 b. temperature control and (based on risk assessment) humidity control;
4274 c. a filtered air supply that maintains a pressure differential and airflow to adjacent cleanrooms
4275 of different cleanliness levels to prevent reversal of airflow direction between the segregated
4276 cleanrooms. A combination of negative and positive pressure can also be used to achieve
4277 specific biosafety requirements;
4278 d. a documented system for monitoring temperature, humidity, air-supply conditions, pressure
4279 differentials, viable and non-viable particle numbers (for environmental monitoring, see
4280 below at §7.5.2);
4281 e. a documented system for cleaning and disinfecting cleanrooms and equipment;
4282 f. a documented system for gowning and laundry;
4283 g. adequate space for personnel to carry out their operations;

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h. adequate space for storage of sterile garments;
i. access limited to authorised personnel.

Table 7.1. Air cleanliness classifications in Europe

Classification		**Maximal number of particles/m³**			
ISO 14644-1	EU GMP	ISO 14644-1		EU GMP	
				at rest	in operation
		≥0.5 µm	≥5.0 µm	≥0.5 µm	≥0.5 µm
ISO 5	A	3 520		3 520	3 520
	B			3 520	352 000
ISO 6		35 200	293		
ISO 7	C	352 000	2 930	352 000	3 520 000
ISO 8	D	3 520 000	29 300	3 520 000	not defined

Source: [1, 2].

Characteristics such as temperature and relative humidity are dependent on several factors (air changes in the room, number of personnel, heat load of the equipment, processing methods and external influences such as weather changes). Parameter settings should not interfere with the defined cleanliness levels. The environmental temperature and relative humidity should be set to guarantee the safety and quality of the tissue and cells, staff comfort, electrostatic charging and discharge. Energy consumption can also be taken into account. For relative humidity, the generally accepted guidance range is 30 % to 65 % (ISO 14644-4) [3].

To minimise the risk of contamination, a positive pressure should be created relative to adjacent cleanrooms of a lower grade. The pressure differential between adjacent cleanrooms of different grades should be 10-15 Pa (guidance values in GMP, Annex 1) with the maximal air pressure in the background environment for the Grade A zone (the working room in Figure 7.1) [1]. This forms a 'pressure cascade' to prevent reversal of airflow direction between the segregated cleanrooms and limits the entry of contamination into the cleanrooms of a higher cleanliness level.

Stringent biosafety requirements should be followed if processing tissues or cells from patients having known viral infections, e.g. hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV). The required precautionary activities or the need for a special contained laboratory (a processing room having reduced air pressure relative to the adjacent rooms) should be determined by documented risk analyses. Risk analyses should consider risks relating to cross-contamination of other tissues and cells processed at the tissue establishment. In addition, risk analyses should consider personnel safety according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work [4].

However, when working with viruses (see Chapter 30) the cleanroom installation must also protect the environment, and therefore the processing room should have reduced air pressure relative to adjacent rooms (contained laboratories at biosafety level 2 and biosafety level 3). A possible solution is to increase the air pressure in the change rooms to result in the working room having reduced air pressure with respect to the last change room (see Figure 7.1). Another specific safety consideration when working with viruses for genetic cell modifications is the protection of the worker. For this reason the use of biosafety cabinets is mandatory. In many cases Class II biosafety cabinets are sufficient but, depending on the virus present, an isolator cabinet is recommended.

Residue manipulation is another issue when working with viruses. To protect the environment all material must be autoclaved before leaving P2/P3 (biosafety level 2/3) contained laboratories. The use of autoclaves inside these laboratories, or in between contained laboratories and the next room, is common. However, validating autoclaves in processing facilities is complicated by the use of water. One possible solution is to place all residues in hermetic containers and autoclave them in another room of the facility. If this is the case, these procedures should be validated.

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Figure 7.1. Schematic plans indicating air pressure differentials between adjacent cleanrooms

Typical processing facility design | Biohazard processing facility design

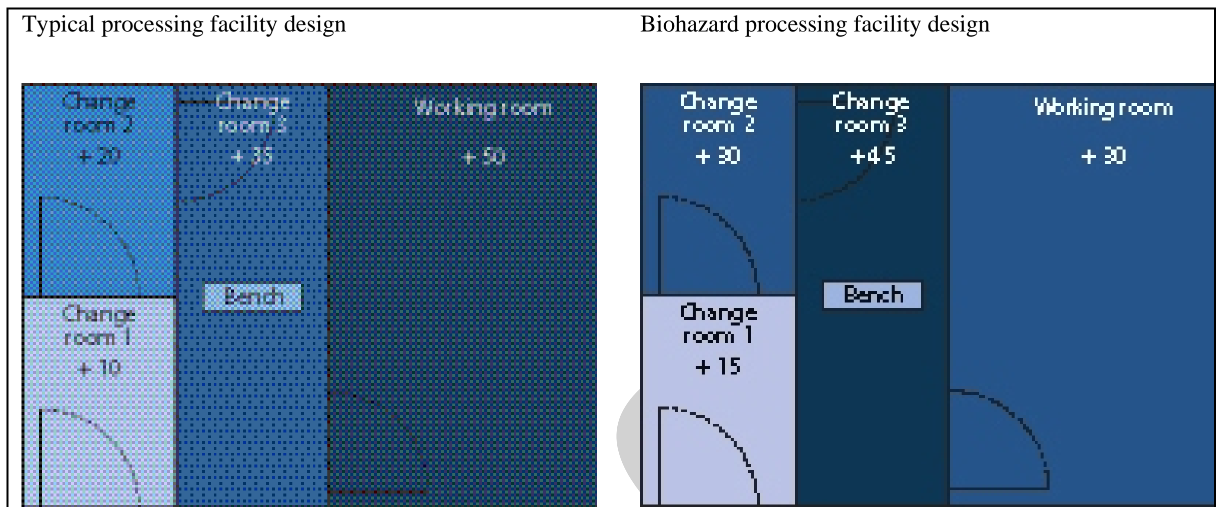

Figure 7.1 shows schematic plans indicating the air-pressure differentials between adjacent cleanrooms of a processing facility for conventional or biohazard use. Conventional processing facilities are designed to protect the sample from any contamination and therefore there is an air-pressure increase in subsequent rooms of 10-15 Pa, with the maximum air pressure in the processing room. However, biohazard-processing facilities must be designed to protect both the tissue and cells and the environment. Therefore the installation must increase the air pressure in the change rooms, resulting in maximum air pressure in one of the change rooms, and the air pressure of the working room being less than in this change room.

7.4. Selecting the appropriate air quality for processing

According to GMP, aseptic processing must be done in a Grade A zone within a Grade B background environment. For tissue and cell establishments in the EU, there must be the equivalent of Grade A with a surrounding environment of at least Grade D (GMP classification). A less stringent processing environment may be acceptable if one of the following applies:

a. a validated microbial inactivation or validated terminal sterilisation process is applied; or,
b. if it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissues or cells concerned; or,
c. if it is demonstrated that the mode and route of application of the tissues or cells to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with transplantation of tissues and cells; or,
d. if it is not technically possible to carry out the required process in a Grade A environment – e.g. due to the requirements for specific equipment in the processing area that is not fully compatible with Grade A (Directive 2006/86/EC) [5].

Many national requirements are more stringent, requiring Grade A with a surrounding environment B or C for certain processes.

As outlined in Table 7.2 the specification of the air quality of the processing environment should be decided on the basis of the particular types of tissue or cell and the processing method that is being applied. Based on a risk assessment, several factors (such as limitations of sampling methods, contamination during manipulation, use of antimicrobials, transfer of contaminants at transplantation) should be taken into consideration when determining the air-quality specifications, especially when less stringent conditions are applied. Where the risk of tissue or cell contamination during processing is high,

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4357 and the chances of any contaminants being transferred to the recipient are high, a more stringent air-
4358 quality specification should be adopted. See also Chapter 8 and the tissue-specific recommendations
4359 provided in part B of this guide.
4360

4361 **Table 7.2. Risks of contamination that should be considered when determining air-quality specifications of**
4362 **processing facilities**

Risk	Explanation
Tissue or cell contamination during open *versus* closed processing	Processes that are functionally 'closed' need a less stringent processing environment than processes where tissue and cells are exposed to the environment
Effectiveness of the processing method to remove contaminants	Some tissues, even though not terminally sterilised, can be treated with various antimicrobial agents; this reduces the risks of transferring any contaminants
Suboptimal detection of contaminants due to the sampling method	If the only option for final microbiological sampling is swabbing or testing of unrepresentative samples, the risk that contaminants will be undetected is higher than in processes where 5-10 % destructive testing of final tissue and cell grafts can be performed [6]
Transfer of contaminants at transplantation	Tissues that are minimally processed, or cellularised, or contain blood, blood vessels and lipids are more likely to support microbial contaminants than those that are blood- and cell-depleted Method of application (i.e. permanent *versus* temporary) and site of transplantation both affect the risk of transfer of contaminants
Source: Euro GTP Hot Topics guidance [7].	

4363
4364 The utilisation of isolator technology to minimise human interventions in processing areas may
4365 result in a significant decrease in the risk of microbiological contamination of aseptically manufactured
4366 tissue and cells. The air classification required for the background environment depends on the design
4367 of the isolator and its application. It should be controlled, and for aseptic processing it should be at least
4368 Grade D. Isolators should be used only after appropriate qualification. Qualification should take into
4369 account all critical factors of isolator technology, for example the quality of the air inside and outside
4370 (background) the isolator, sanitisation of the isolator, the transfer process and isolator integrity (checking
4371 for defective seals and pinhole leaks in the isolator gloves).

4372 7.5. Qualification and monitoring

4373 Processing environments and surrounding areas must be qualified and monitored in accordance with EN
4374 ISO 14644 [2, 3, 8, 9], EN ISO 14698 [10] and EU GMP Annex 1 [1]. The validation master plan should
4375 be built following a risk-assessment exercise aiming to define the extent and frequency of the
4376 qualification tests in a proportional manner to the risks identified. The validation plan should consider
4377 the initial and consequent qualification, as well as the at-rest and in-operation classification (see also
4378 Chapter 2). An example of a validation master plan is provided in Appendix 23 of this guide.
4379 The qualification strategy should consider the monitoring strategy displayed over the process.
4380 Whenever a particular process is submitted to a continuous air-quality monitoring program, the
4381 frequency of qualification of cleanrooms, laminar airflow work stations, isolators and RABS might be
4382 adapted accordingly to a less stringent program.

4383 7.5.1. Qualification

4384 Qualification of cleanrooms and clean zones is required to support and verify the operating
4385 parameters and limits for the critical parameters. The specified acceptance criteria set should be verified,
4386 and therefore testing of certain parameters and specifications should be performed. The classification is
4387 part of the qualification of cleanrooms and clean zones and should be clearly differentiated from
4388 monitoring operational processes.

4389 *7.5.1.1. Classification*

4390 Classification is a method of assessing the level of air cleanliness against a specification for a
4391 cleanroom or clean-zone device by measuring the airborne particle concentration. For classification, the

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4392 required tests and acceptance criteria should be defined in the approved classification protocol.
4393 Classification should be performed at rest and in the operational state.
4394 For particle count, the minimum number of sampling locations related to the area of each
4395 cleanroom or clean zone to be classified is provided in EN ISO 14644-1. It divides the whole cleanroom
4396 or clean zone in sections of equal area and selects in each section a sampling location considered to be
4397 representative of the characteristics of the section. The position of the particle probe should be located
4398 at the same height and in the plane of the work activity.
4399 The particle counter must have a valid calibration certificate. The frequency and method of
4400 calibration should comply with the requirement of ISO 21501-4 [12].
4401 For classification, the airborne particles equal or greater than 0.5 µm should be measured. This
4402 measurement should be performed both at rest and in operation. Classification in operation may be
4403 carried out during routine or simulated operations, with a specified number of personnel present.
4404 The minimum air-sample volume per sampling location should be determined in accordance with
4405 EN ISO 14644-1 Annex A. Sequential sample techniques could be useful to classify a cleanroom or clean
4406 zone with a very low particle concentration at the class limit (EN ISO 14644-1 Annex D).
4407 The cleanroom or clean zone has met the specified classification if the particle concentration
4408 measured at each of the sampling locations does not exceed the concentration limits as defined in the
4409 classification protocol.
4410 Periodic classification testing should be performed annually in accordance with ISO 14644-1. This
4411 frequency can be extended, based on risk assessment, the extent of the monitoring system and data that
4412 are consistently in compliance with acceptance limits or levels defined in the monitoring plan.

4413 *7.5.1.2. Other qualification tests*

4414 Table 7.3 specifies optional test methods characterising the performance of cleanrooms and clean
4415 zones. The choice of tests should be based on factors such as the design of the installation, operational
4416 states and the required level of air cleanliness. The selected tests should be repeated as specified in Table
4417 7.3 as a part of validation master plan. Deviations from the pre-set frequencies should be based on a
4418 formal risk assessment. All these tests should be undertaken by qualified professionals at least in an at-
4419 rest situation in accordance with EN ISO 14644-3 [9] which specifies ancillary tests related to other
4420 aspects of cleanroom performance such as pressure difference and airflow. The microbial load of the
4421 cleanroom should be determined in operational state as part of the cleanroom qualification. The
4422 recommended maximum limits for microbial contamination during qualification for each grade are
4423 given in Table 7.5.
4424 Biohazard laminar-airflow hoods should also be certified to national or international performance
4425 standards at the time of installation and recertified annually.

4426 **Table 7.3. Qualification tests for cleanrooms, clean zones and laminar-flow hoods**

Tests	**Specification**	**Recommended time interval**
Airborne particle count (classification test)	Total count of airborne particles (viable and non-viable) performed at rest and in operation, to determine cleanliness class	12 months
Airflow test	Average airflow velocity and air changes per hour	12 months
Air pressure difference	Differential pressure between different rooms	12 months
Installed filter system leakage test	Detection of leaks in the absolute filter and integrity testing of seals between filter and mounting arrangements	24 months or if resistance across filter changes abnormally
Temperature and relative humidity		12 months
Recovery test	Time required for a cleanroom to recover after a particle-generation event – normally tested for cleanrooms classified as Grade A or B; maximum delay given by GMP Annex 1 is 15-20 min	24 months
Airflow direction test and visualisation	Airflow pattern type, i.e. unidirectional, non-unidirectional or mixed	24 months
Containment leak test	Detection of leaks on structure	24 months
Laminar airflow velocity (laminar flow hoods)	The average velocity must meet the specified acceptance criteria	12 months

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Microbial contamination	The total count of viable particles performed in operation	12 months

7.5.2. Monitoring particle concentration

Monitoring particle concentration provides evidence of continuous compliance with the specified air-cleanliness class. ISO 14644-2 specifies minimum requirements for a monitoring plan for a cleanroom, related to air cleanliness by particle concentration.

Cleanrooms and clean zones should be monitored while in operation. Measuring locations should be determined on the basis of a formal risk analysis and the results obtained during qualification of cleanrooms. A monitoring plan, taking into consideration the level of air cleanliness specified, critical care points and performance attributes of the cleanroom, should be created and maintained.

Adequate alert and action limits should be set, based on the intent and purpose of monitoring, taking into account the nature of the process. For example, bone cutting may generate numerous particles, and corneal lamellar cutting may generate numerous aerosols. If the alert limits are exceeded, further investigation or increased observation are required. If the action limits are exceeded, appropriate corrective actions should be taken. Frequent and continuous high particle counts should raise concerns because they may indicate the possibility of pollution, problems with a heating, ventilating and air-conditioning (HVAC) system, or incorrect practices during routine operations. The performance of the monitoring systems and related trends should be periodically reviewed.

Monitoring may be continuous, sequential or periodic (indicating specified frequency).

The system selected must be adequate for the monitoring operations required. If using sequential systems, particle losses because of the length of the tubes and kinks in the tubing should be considered. For airborne-particle counters, the frequency and method of calibration should be based upon current accepted practice as specified in ISO 21501-4 [11].

Selection of the particle monitoring system should also involve consideration of the risks generated by sampling during processing. The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of cleanrooms and clean-air devices.

The Grade A zone should be monitored with a frequency that allows detection of sporadic increases in particle counts which may exceed acceptable limits. It is recommended that a similar system be used for Grade B zones, though the sample frequency may be decreased. The importance of the particle-monitoring system should be determined by the effectiveness of the segregation between adjacent Grade A and B zones.

Table 7.4. Recommended limits for airborne particle concentration during monitoring

Monitoring		**Recommended maximal number of particles/m³**					
ISO 14644-1	EU GMP	ISO 14644-1		EU GMP			
				at rest		in operation	
	Grade	≥0.5 µm	≥5.0 µm	≥0.5 µm	>5.0 µm	≥0.5 µm	>0.5 µm
ISO 5	A	3 520		3 520	20	3 520	20
	B			3 520	29	352 000	2 900
ISO 6		35 200	293				
ISO 7	C	352 000	2 930	352 000	2 900	3 520 000	29 000
ISO 8	D	3 520 000	29 300	3 520 000	29 000	Set a limit based on the risk assessment	Set a limit based on the risk assessment

The particle limits given in Table 7.4 for the at-rest state should be achieved after a short recovery time period of 15-20 min in an unmanned state after completion of operations.

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4464 Monitoring of Grade C and D areas should be performed in operation and in accordance with the
4465 principles of quality risk management.
4466 Temperature, relative humidity and differential pressure of clean areas should be monitored every
4467 day.

4468 **7.5.3. Microbiological monitoring**

4469 Microbiological monitoring provides evidence of continuous compliance with the specified air-
4470 cleanliness class as well as evidence of contamination control of aseptic process operations and cleaning
4471 and sanitation methods. Microbiological monitoring is mandatory and should be done in accordance
4472 with:
4473 a. EN ISO 14698 *Cleanrooms and associated controlled environments – Biocontamination control*
4474 [10]; and
4475 b. EU GMP Annex 1.
4476 To define and control microbiological hazards it is necessary to identify the potential risks relating
4477 to each processing step and the potential risks of the tissues or cells themselves, as well as the probability
4478 of these risks and the mitigation actions intended to minimise the risks. Tissue establishments must have
4479 a monitoring plan that specifies:
4480 a. acceptance limits of microbial contamination (action level, alert level);
4481 b. sampling plan and frequency;
4482 c. sampling methods and equipment (see Chapter 10);
4483 d. sampling culture media and incubation of samples (see Chapter 10);
4484 e. analyses and evaluation of results (including trend analyses);
4485 f. handling of out-of-specification results.
4486 Selection of the microbial monitoring method should involve consideration of the risks generated
4487 by sampling during processing. The sample sizes taken for monitoring purposes will usually be a
4488 function of the sampling rate of the system used. It is not necessary for the sample volume to be the
4489 same as that used for formal qualification of cleanrooms and clean-air devices.
4490 The frequency of sampling should take into account the processes and activities of the staff.
4491 Aseptic process operations performed in a Grade A or B environment should be monitored routinely.
4492 Background and surrounding areas could be monitored periodically.
4493 Results of monitoring should be considered when making the decision whether tissues or cells
4494 can be released (Figure 7.2).
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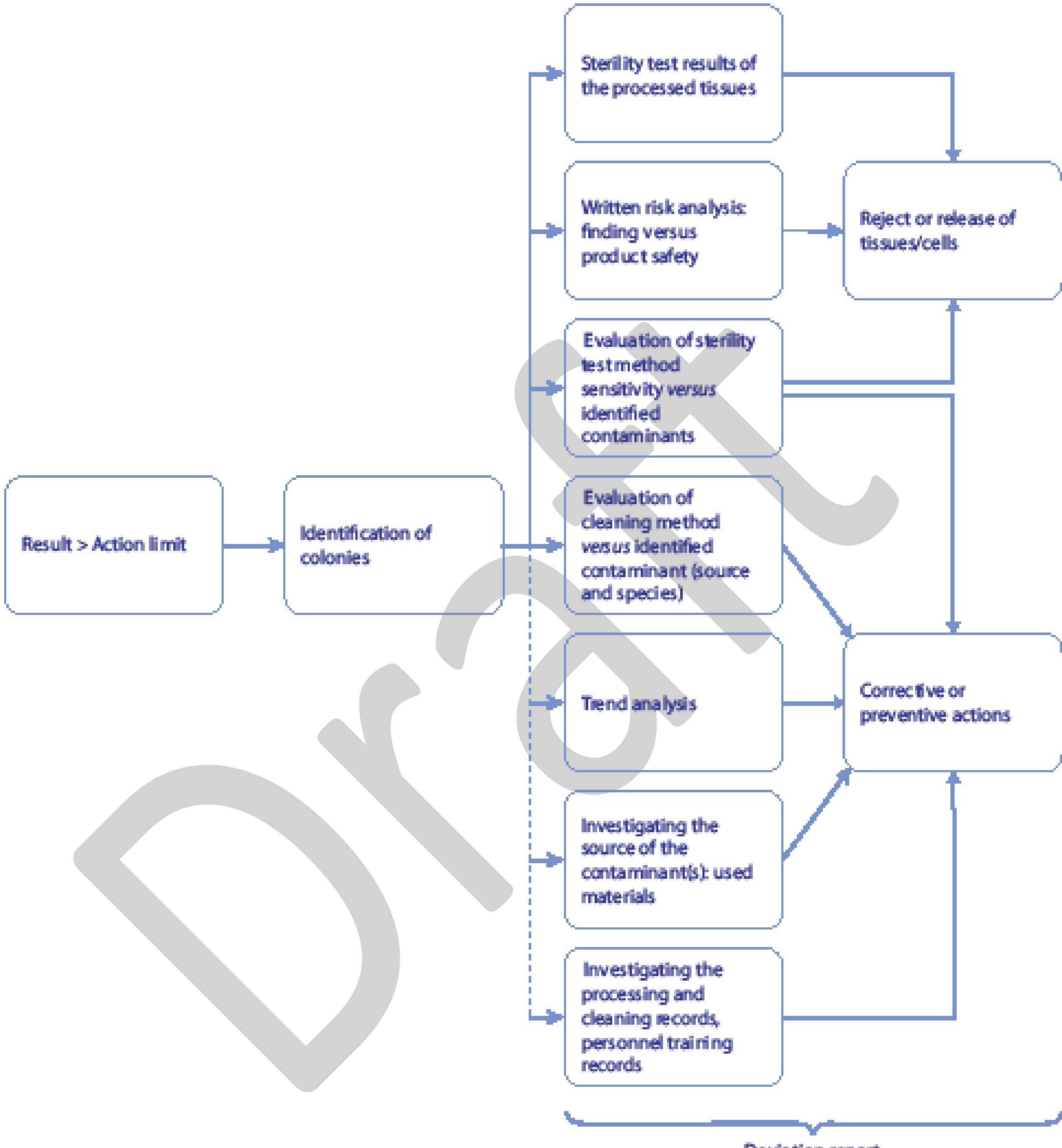
4496 **Figure 7.2. Decision tree: topics and actions to be considered if microbiological-monitoring results**
4497 **(number of colonies) exceed the action limit**



4498
4499 Note: In Grade A and B areas, detected colonies must be identified by genus and species, and for other cleanliness according to the
4500 specifications of the microbial monitoring programme. Solid lines indicate minimal actions to be considered, and dashed lines indicate topics
4501 of broader investigations.
4502
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Table 7.5. Recommended limits for microbial monitoring (EU GMP Annex 1)

	Recommended limits for microbial contamination			
Grade	***Air sample (cfu/m³)***	***Settle plates, diam. 90 mm (cfu/4 hours)[(a)]***	***Contact plates, diam. 55 mm (cfu/plate)***	***Glove print, 5 fingers (cfu/glove)***
A[(b)]	1	1	1	1
B	10	5	5	5
C	100	50	25	not applicable
D	200	100	50	not applicable

a. Individual settle plates may be exposed for less than 4 hours. Where settle plates are exposed for less than 4 hours the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 hours.

b. Note that for Grade A the expected result should be 0 cfu recovered; any recovery of 1 cfu or greater should result in an investigation.

Recommended limits for microbiological monitoring of clean areas during operation are shown in Table 7.5. Alert and action levels for microbial contamination should be determined and the actions to be taken in the event that these levels are exceeded should be documented. The level should be specific to the area, determined on the basis of historical data and based on, for example, data from a single year. Levels should be reviewed periodically and, if necessary, updated if there are changes to processes.

The alert level emphasises an acceptable number for microbial contamination, but acts as a warning. Exceeding the alert level does not require corrective measures, but should trigger an investigation aimed at early detection of errors or deviations. The alert level is set at a lower level than the action limit. The action level emphasises a certain level of microbial contamination that necessitates immediate corrective action and corrective measures.

In Grade A and B areas, detected colonies must be identified by the genus and species, and for other cleanliness grades according to the microbial monitoring programme of the tissue establishment. Figure 7.2 describes topics and actions to be considered if microbiological-monitoring results exceed the action limit. Any presence of fungi or yeasts must be considered to denote deviation and should be identified.

After such results are obtained, tissue establishments should evaluate whether the finding will affect the risk that tissues or cells could have been contaminated during processing. Tissue establishments should also evaluate whether corrective or preventive actions should be initiated. All investigations that are carried out should be reported in a deviation report.

7.6. Avoiding contamination and cross-contamination

Entry of personnel and materials to the processing facilities, transit and exit of personnel and material through the processing area and the rules of use and clothing to be worn in them should be established to:

a. minimise the risk of contamination of tissues and cells;
b. reduce the environmental bioburden;
c. protect staff from biohazards.

A written procedure designed to avoid potential contamination and/or cross-contamination from personnel and materials to tissues and cells should be in place.

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Entry of personnel, tissues and cells and materials should be done through airlocks by following specified procedures to avoid the direct flow of non-treated air into cleanrooms. Both airlock doors should not be open simultaneously. An interlocking system or a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.

Only the minimum number of personnel required for efficient processing should enter the processing areas. The need for additional persons to be present in processing areas should be taken into account during risk assessment when the procedure is being designed.

High standards of personal hygiene and cleanliness are essential. Changing and washing must follow a written procedure designed to minimise contamination of clean area clothing or transfer of contaminants to the cleanrooms. Wrist-watches, make-up and jewellery must not be worn in clean areas. Outdoor clothing must not be brought into changing rooms that lead to Grade B and C rooms.

Required clothing should be chosen, based on the process and grade of the processing area. Minimum requirements modified from EU GMP Annex 1 are listed in Table 7.6. Clothing should be pocket-less, made of lint-free material, with tightly fitting fasteners at the neck, wrist and ankles. For each worker in a Grade A/B area, clean sterile (sterilised or sanitised adequately) protective garments should normally be provided at each work session (or slightly less often if monitoring results justify it) but in any case at least once a day. Masks and gloves should be changed at least after each working session. Gloves should be changed regularly during operations. Disinfection of gloves is acceptable in Grade C and D environments as long as direct contact with tissues and cells is excluded. When operators are trained in the use of good aseptic practices, documented with a successful process simulation, the operator glove sites can be considered as a non-critical surface [12]. The efficacy of disinfection depends on the disinfectant/type of gloves combination. Disinfection of some gloves has an influence on the tightness of the gloves [13]. A complete investigation should be performed and documented to evaluate the impact of glove disinfection.

Gowning procedures for personnel should be validated at least in Grade A/B areas to ensure that gowning materials and protocols are adequate. Samples should be taken from the surface in several fixed sites on clothing:

a. fingers of gloves;
b. facemask;
c. sleeve (forearm) of a suit;
d. front of the suit at chest height;
e. hood.

Table 7.6. Minimum clothing requirements (adapted from EU GMP Annex 1)

Classification	Clothing	Description
Grade D	Facemask	Depending on the process, at least beards and moustaches should be covered
	Cap	Hair should be covered
	Suit	A general protective suit
	Shoes	disinfected or sterilised shoes or overshoes
	Gloves	Dependent upon the process
Grade C [(a)]	Facemask	Depending on the process, at least beards and moustaches should be covered
	Cap	Hair should be covered
	Suit	A single or two-piece trouser suit gathered at the wrists and with high neck
	Shoes	disinfected or sterilised shoes or overshoes
	Gloves	Sterile, non-powdered rubber or plastic gloves
Grade A/B [(a)]	Facemask	Sterile, single-use. Headgear should totally enclose facial hair. Sterile eye protection/coverage is dependent upon the process
	Cap	Sterile headgear should totally cover hair, beards and moustaches; it should be tucked into the neck of the suit
	Suit	Sterile coverall
	Shoes	Sterilised footwear, boot-like structure to enable the trouser-legs to be tucked inside the footwear
	Gloves	Sterile, non-powdered rubber or plastic gloves
a. In general, the protective clothing material should shed no fibres, and clothing should retain the particles shed by the body		

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7.7. Cleaning

Appropriate sanitation of clean areas is of the utmost importance to satisfy environmental requirements. The cleaning process (both schedule and procedure) should be validated, and the validated cleaning process should be followed to achieve the required level of cleanliness. The cleaning validation should consider the influence of the time between processing and cleaning and the time between cleaning and processing to define how long the cleaning process can be delayed after processing and how long the cleaning process remains effective. All cleaning procedures should be documented. Cleaning should be done by personnel trained for the procedure, cleanroom environment, workflows and gowning. The rotation of disinfectants should be included in the disinfection programme to avoid any antibiotic resistance effect due to biocides [14] and to cover all the range of micro-organisms. Cleaning products are made up of broad-spectrum disinfectants containing quaternary ammonium compounds, stabilised chlorine dioxide, hydrogen peroxide and sodium hypochlorite.

Certain cleaning products might be detrimental for certain tissues and cells. Cleaning products should therefore not only disinfect the premises but also be safe for the human tissues and cells. Especially in ART centres, certain biocides might be detrimental for gametes and embryos, and therefore care should be taken in choosing the appropriate cleaning products and disinfectants [15]. Disinfectants and detergents used in Grade A and B areas should be sterile before use. Microbiological monitoring of the cleanroom should be undertaken regularly to detect development of resistant strains. Fumigation may be useful for reducing microbiological contamination on inaccessible surfaces.

Some tissue banks and cell banks accept material for autologous use from donors infected with HIV, HBV or HCV. In such cases, separate processing should be done and validated cleaning procedures applied. After processing, the surface should be decontaminated using disinfectant with specific label claims for blood-borne pathogens (e.g. HIV, HBV, HCV) or a freshly diluted bleach-based product in accordance with manufacturer’s instructions, and the surface should be allowed to dry.

Inactivation of prions should be considered if risk of prion contamination has occurred, e.g. if tissues or cells from a Creutzfeldt–Jakob disease-positive donor have been processed or stored. Prions are very resistant to inactivation. Published methods for prion inactivation include physical and chemical methods. Concentrated solutions of sodium hypochlorite achieve inactivation but other chlorine-releasing compounds are less effective. Sodium hydroxide (2 M) leads to substantial (but incomplete) inactivation. Other chemical procedures, such as use of proprietary phenolic disinfectants, are much less effective. Infectivity can survive autoclaving at 132-138 °C and, under certain conditions, the effectiveness of autoclaving declines as the temperature is increased. The small resistant subpopulations that survive autoclaving are not inactivated simply by re-autoclaving, and they acquire biological characteristics that differentiate them from the main population. Despite the limitations of autoclaving, combining autoclaving (even at 121 °C) with treatment using sodium hydroxide is extremely effective [16].

Storage facilities should be cleaned according to a schedule. Also, handling and disposal of wastes should include appropriate collection, storage and transportation procedures according to applicable European, national and local regulation.

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Chapter 8. Processing

8.1. Introduction

'Processing' means all operations involved in the preparation, manipulation, preservation, packaging and inactivation of micro-organisms in tissues or cells intended for human application. Storage is necessary at various stages from procurement to clinical use and must be controlled and documented to ensure that the required properties of the tissues or cells are maintained and that cross-contamination or loss of traceability is avoided (see Chapter 9, Storage and release). Packaging and labelling are described in more detail in Chapter 14.

The aims of processing tissues and cells include:

a. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of tissues or cells;
b. preservation of the required properties of the biological material, making extended storage for future use possible;
c. reducing the risk of disease transmission by the inactivation of micro-organisms or even sterilisation in circumstances where cell viability is not required;
d. improving the clinical performance of a graft by removing those elements that are not necessary for the success of the transplant.

Processing includes a range of activities such as (but not limited to) thawing, washing, cutting, grinding, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, separation, decellularisation, concentration or purification of cells, freeze drying, freezing and cryopreservation.

Although it can deliver great benefits, processing can also introduce risks. The potential risks include:

- microbial contamination from the environment or the operator, or cross-contamination from other tissues or cells,
- errors in identification or labelling,
- damage to the tissues or cells which reduces their clinical efficacy.

For these reasons, processing of tissues and cells must be carried out within a comprehensive quality-management system, must be documented using standard operating procedures (SOPs) and must be thoroughly validated, to demonstrate that the quality and efficacy of the final product have not been unacceptably compromised and that contamination or cross-contamination has not been introduced during processing.

Procurement is defined as the technique for obtaining different tissues directly from the donor, e.g. procurement of corneas by *in situ* excision or procurement of menisci by arthrotomy. However, if the eye was removed from the donor, and corneas and menisci were then excised at the procurement site, this activity would be classified as processing. Processing in the procurement facility, either during or after procurement, is not recommended because it is important to prevent microbial contamination, or cross-contamination of procured tissues. (See Chapter 6 on Procurement.) This chapter provides generic guidance on the processing of tissues and cells carried out by tissue establishments. Further, more specific, guidance is provided in Part B of this Guide. It is also important that the Good Practice Guidelines for Tissue Establishments (GPG) that follow EU directives are complied with. Where relevant, the GPG sections pertinent to the topic are referenced in each subsection of this chapter.

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8.2. Receipt at the tissue establishment

Each tissue establishment (TE) must have a documented policy and specifications against which each consignment of tissues and cells (including blood samples from donors) is verified. These specifications must include the technical requirements and other criteria considered by the TE to be essential for the maintenance of acceptable quality. When the procured tissues or cells arrive at the TE, there must be documented verification of the consignment. Documents must be completed covering the transport – including the transport conditions, packaging, labelling and associated documentation – and samples (including blood) to ensure that they meet the requirements and specifications of the receiving establishment (and, in EU countries, the requirements of Annex IV of Commission Directive 2006/17/EC).

Upon receipt of the documentation, the procurement report and shipping record (if the donation was transported by a third party) should be cross-checked with the contents of the package. The packaging, the tissues and cells received, and any accompanying samples should all be examined to ensure that they have not been damaged or tampered with during transit.

The following should be checked and recorded:

a. (no) evidence of unauthorised opening or manipulation;
b. (no) signs of damage that might result in the deterioration of tissues and cells and (no) signs of incidents relating to storage;
c. transport conditions (unless a validated transport method has been used) and storage temperature and time in transit;
d. identification of the donor (donation number);
e. description of the tissues or cells (including number of units per device or ampoule);
f. procurement report including procurement date and time;
g. purpose of tissues and cells (i.e. for transplant/research);
h. status of the tissues or cells (e.g. quarantine);
i. associated samples (including blood).

The TE must ensure that the tissues and cells received are quarantined and stored in a defined, separated and adequate location under appropriate conditions until they, along with the associated documentation, have been inspected or otherwise verified as conforming to requirements. The acceptance or rejection of received tissues or cells must be documented.

The data that must be registered at the TE include:

a. consent/authorisation, including the purpose(s) for which the tissues and cells may be used and any specific instructions for disposal if the tissues or cells are not used for the purpose for which consent was obtained;
b. all required records relating to the procurement and donor medical/behavioural history (see §6.5);
c. for allogeneic donors, eligibility, i.e. a properly documented review of donor evaluation against the appropriate selection criteria by an authorised and trained person;
d. in the case of tissues and cells intended for autologous use, documentation of the possibility of medicinal allergies (such as to antibiotics) of the recipient.

Review of the relevant donor/procurement information, and thus acceptance of the donation, needs to be carried out by specified/authorised persons.

The TE must have documented procedures for the management and segregation of non-conforming tissues or cells, or those with incomplete test results for infectious diseases, to ensure that there is no risk of contamination of other tissues and cells being processed, preserved or stored.

If the material is not being transported by their own personnel, the TE should prepare an agreement to be signed by third parties that defines the responsibilities of each party in the transport of tissues and cells to the TE. Such transport should be direct and without intermediate stops where possible, to ensure the safety and maintenance of the temperature conditions of the tissues and cells and prevent unauthorised access.

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4737 Quality-control checks of procurement and transportation methods should be reviewed regularly
4738 by TEs to ensure that the integrity of tissues or cells and the storage temperatures are maintained during
4739 procurement and transit.

4740 8.3. Coding

4741 TEs must ensure that human tissues and cells are correctly identified at all times. Upon receipt of the
4742 tissues and cells, the TE should assign a unique identification code to the material if this has not already
4743 been done at procurement. This code can then be extended to identify the different products and batches
4744 of tissues or cells obtained during processing.
4745 Tissues and cells should be labelled at all stages of processing (see Chapter 14 for further guidance
4746 on labelling and Chapter 15 for further guidance on traceability). The label must include at least the
4747 following information:
4748 a. unique identification;
4749 b. identification of the TE;
4750 c. type and characteristic of the product;
4751 d. batch number (if applicable);
4752 e. recipient name (if applicable).

4753 The coded data must be entered in a register maintained for the purpose.

4754 8.4. Processing methods

4755 Tissues and cells should be appropriately processed, preserved and decontaminated for clinical use. TEs
4756 must address all processes that affect quality and safety through their quality system and associated
4757 standard operating protocols (SOPs).
4758 TEs must ensure that the equipment being used, the working environment, process design, and
4759 validation and control conditions are in compliance with established quality and safety requirements
4760 (see Chapter 2). Each step of processing must be carried out under defined conditions to guarantee the
4761 quality and safety of tissues and cells, as well as the safety of TE personnel.
4762 If a TE entrusts one of the stages of processing to a third party, a written agreement is needed
4763 between the TE and the third party. The TE must evaluate and select third parties on the basis of their
4764 ability to meet the established standards.
4765 The recommended time limits between procurement, processing and storage are described in the
4766 tissue- and cell-specific sections of this Guide (see Part B). When appropriate, these maximum times
4767 from procurement (or circulatory arrest) until processing and storage must be defined. Procurement,
4768 processing and storage times must be documented in the records for tissues and cells.

4769 8.4.1. Processing reagents

4770 The reagents used in processing should be of an appropriate grade for their intended use, be sterile
4771 (if applicable) and comply with existing national regulations. Use of antibiotics during procurement,
4772 processing and preservation should be minimised, and, if used, information regarding the possibility of
4773 allergic reactions in the recipient must be included in the information provided to the end users.
4774 Whenever possible, reagents used for procurement, processing, decontamination and preservation
4775 should be approved for human use and should be CE (Conformité Européenne) marked. Reagents that
4776 are not of appropriate grade must undergo risk assessment and validation to confirm that they are suitable
4777 for their intended purpose. Reagents not approved for human use may be used if an equivalent reagent
4778 of appropriate grade is not available, if the use has been authorised by national authorities and if the use
4779 is supported by risk assessment. The origin, characteristic conditions for storage (physical, chemical,
4780 microbiological) and expiry dates of reagents should be monitored and recorded. Reagents should be
4781 used in a manner consistent with the instructions provided by the manufacturer. Critical reagents and
4782 consumables should have written specifications describing, if applicable:

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4783 a. materials, including:
4784 i. the designated name and the internal code reference;
4785 ii. the reference (if any) to a pharmacopoeia;
4786 iii. the approved suppliers and, if possible, the original manufacturer of the products;
4787 iv. a specimen of printed materials;
4788 v. certificate of compliance from the manufacturer.
4789 b. directions for sampling and testing, or reference to procedures;
4790 c. critical quality attributes, with acceptance limits;
4791 d. storage conditions and precautions;
4792 e. the maximum period of storage permitted before re-examination.

4793 **8.4.2. Processing techniques**

4794 *8.4.2.1. General principles*

4795 Processing methods must not render the tissues or cells clinically ineffective or harmful to the
4796 recipient. They should be designed to ensure the safety and biological functionality of prepared tissues
4797 and cells. Processing methods should be validated to ensure they achieve their objectives (see the general
4798 text on validation in Chapter 2 and §8.10 below).
4799 Processing procedures must undergo regular re-validation to ensure that they continue to achieve
4800 the intended results.

4801 Pooling of different tissues and cells from two or more donors during processing is not
4802 recommended. The only exception is where it is supported by a comprehensive risk–benefit assessment
4803 and it has been demonstrated to be the only way of providing sufficient clinically effective tissues or
4804 cells. If performed, traceability must be fully ensured.

4805 *8.4.2.2. Procedures*

4806 The main types of processing procedure that can be applied to tissues and cells include, but are
4807 not limited to:
4808 • Cleansing of procured material by removal of extraneous tissues and bodily fluids is a common
4809 initial processing step. Commonly, scalpels, scissors and gauze wipes are used in this process.
4810 • Separation is used to partition the specific type of tissue to be processed from another type, for
4811 example to divide dermis and epidermis, or amniotic membrane and chorion.
4812 • Cutting and Shaping allows initial preparation of procured tissues into the shapes and forms
4813 required for transplantation. Different types of cutting device can be used, depending on type
4814 of tissue. For cutting bone, different types of saw may be used, such as oscillating saws,
4815 bandsaws or rotary saws, whereas for soft tissues such as skin and tendon, scissors or scalpels
4816 may be used.
4817 • Grinding, by different types of mill, is used to pulverise bone tissue into smaller pieces.
4818 Depending on the type of bone being ground, actively cooled grinding mills that dissipate heat
4819 may be used, to prevent the bone being damaged by excessive temperatures caused by grinding
4820 friction.
4821 • Washing may be performed in one of three ways:

4822 o as an initial step in processing, e.g. to remove surface blood and lipids,
4823 o as an integral part of a process, e.g. to remove bone-marrow components from
4824 musculoskeletal allografts,
4825 o to remove traces of chemical compounds used during processing.
4826 Washing may also be used to decrease the bioburden of tissues. Several types of washing solution
4827 may be used, e.g. distilled water, 0.9 % NaCl, balanced salt solution, phosphate-buffered saline,
4828 or alcohols. The washing protocol utilised should be validated to demonstrate that it does not
4829 detrimentally affect the clinical efficacy or safety of the tissue [1, 2].

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4830 • Centrifugation may be used to concentrate and separate cells from a suspension or different
4831 fractions of suspensions, or to remove marrow, blood and lipid components from
4832 musculoskeletal allografts.
4833 • Disinfection by soaking or rinsing in antibiotic or antimicrobial solutions is commonly used for
4834 decontamination of viable tissues that cannot be terminally sterilised, and as a stage in the
4835 processing of subsequently sterilised tissues to reduce the bioburden.
4836 • Cell concentration and selection is used as an initial step for HPC processing or for *in vitro* cell
4837 cultures. This may also include the isolation of particular cell types, e.g. mononuclear cells from
4838 peripheral blood.
4839 • Filtering procedure is used after bone-marrow collection.
4840 • Decellularisation is a technique that aims to remove most of the cellular content of the tissue,
4841 leaving behind just the extracellular matrix (ECM). These extracellular matrices may be
4842 implanted directly or used as a scaffold for the manufacture of advanced therapy medicinal
4843 products (ATMPs). See Chapter 31 for a more detailed discussion of decellularisation.
4844 • Demineralisation is a process of chemical removal of the bone mineral, resulting in exposure
4845 of biologically active bone morphogenetic proteins present in bone tissue. Demineralisation is
4846 usually performed using a dilute (0.5 M or 0.6 M) HCl solution.
4847 • Freeze-thawing of tissues can be used as a processing step, for lysis of cells prior to washing
4848 procedures.

4849 8.5. Preservation methods

4850 Preservation of tissues and cells for long-term banking is central to the operation of a TE. It is essential
4851 that a preservation technique appropriate for the graft in question is selected. The selected technique
4852 must be capable of retaining the essential properties of the graft (e.g. viability, structural integrity) for
4853 the duration of the maximum possible storage period.

4854 8.5.1. Types of preservation

4855 Tissues and cells can be preserved by freezing, lyophilisation, cryopreservation, vitrification or
4856 glycerolisation.
4857 Freezing is used for pre-processing storage of procured tissues, for in-processing storage between
4858 different processing steps of non-viable tissues and for storage of processed tissue awaiting release for
4859 transplantation. Freezing can disrupt tissues and cells [3]. Hence, the method of freezing used must take
4860 into account the eventual use of the tissues and cells.
4861 Lyophilisation (or freeze-drying) involves dehydration of tissues by freezing and then reducing
4862 the surrounding pressure to allow the frozen water in tissue to sublimate directly from the solid phase to
4863 the gas phase. Lyophilisation prevents tissue autolysis and allows storage at room temperature.
4864 Cryopreservation is a process where the biological and structural functions of tissues or cells are
4865 preserved by cooling to sub-zero temperatures in a cryoprotectant. This is used where cell viability must
4866 be maintained. The rate of cooling must also be controlled to prevent formation of ice crystals within
4867 cells, which can result in damage and loss of viability and integrity. Once cryoprotectants are added,
4868 tissues/cells may be placed in a freezing device (such as a controlled-rate freezer) that gradually reduces
4869 the temperature of the grafts.
4870 Vitrification is ice-free cryopreservation. The crystallisation of ice is avoided by an extreme
4871 elevation of viscosity during cooling achieved by a combination of high cryoprotectant concentrations
4872 and rapid cooling.
4873 Glycerolisation is a procedure for soaking tissues, mainly skin, in a concentrated glycerol
4874 solution. The glycerol binds water in the tissue and prevents degradative processes occurring.

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8.6. Decontamination methods

Microbiological and viral safety are critical for cell and tissue transplants. Validated sterilisation and virus-inactivation processes are mandatory in many jurisdictions. In some countries a validated disinfection process may be accepted as an alternative to sterilisation.

It is important to clearly define terms when discussing the removal of micro-organisms and the inactivation of viruses. Typically, more than one process contributes to the overall effect. Initial steps generally serve to reduce and/or control the bioburden for the main process.

8.6.1. Types of decontamination

8.6.1.1. Disinfection

Disinfection is a term used for non-sterilising processes that kill bacteria, and/or fungi and/or spores, and/or inactivate viruses via a known, direct and quantifiable physical or chemical mode of action. In the preparation of tissue grafts, disinfection processes are either precursors to sterilisation processes or in some cases, where sterilisation is either not required or not possible, are themselves the primary mechanism for ensuring microbiological graft safety.

Generally, when used as a precursor to sterilisation, a disinfection process is intended to reduce the bioburden on the tissue prior to sterilisation such that it does not exceed a level that can be reliably completely eliminated by the sterilisation process.

When used as the main step for the removal of bacteria, spores and fungi and the inactivation of viruses, the process should be validated according to national requirements. In some instances, sterility tests on individual transplants may be required.

Some establishments rely upon a validated thermodisinfection process for the attainment of microbiological transplant safety [4]. Tissues that cannot be subjected to high temperatures or other disinfection procedures may be treated with antibiotic-based disinfection steps. The risk that antibiotic residues could remain in the tissue post-disinfection, and compromise post-disinfection sterility, must be considered [5]. Such processes should therefore be validated for both efficacy and residual antibiotic levels. It may be necessary to include information about such residues in the information provided to end users, as some patients may be allergic to certain antibiotics.

8.6.1.2. Sterilisation

Sterilisation is defined as a process that results in the complete absence of all cell-based micro-organisms capable of replication. In the preparation of tissue grafts a sterilisation process will usually also have to meet the requirements for virus inactivation.

Sterilisation processes can be based on moist or dry heat, chemical agents, irradiation or high pressure. Sterilisation processes used in tissue-graft preparation are held to exacting standards and must be validated according to national requirements.

Individual validation methods for processes used to sterilise tissue transplants are feasible, but the following established, standardised approaches are available:

a. Sterility Assurance Level (SAL). For sterilisation processes with a well-defined dose/kill relationship, a very high degree of sterility assurance can be achieved and quantified with an SAL. The SAL is expressed as an experimentally-derived number defining the likelihood of a contaminant surviving the process. The smaller the number, the higher the likelihood of sterility. For some classes of medical device and for some medicinal products, there is a European requirement that a sterilisation process must be validated to give a value of SAL $\leq 10^{-6}$ for the product to be labelled "sterile". This SAL means that the likelihood of non-sterility is 1 in 1 million. *(This is often interpreted as meaning that in a theoretical batch with 1 million "units", at least 999 999 units must be sterile. More relevant for tissue transplants is the interpretation that the process should result in all units being sterile in 999 999 from 1 million cycles.)* At the time of writing there are no defined national requirements within Europe for sterilisation processes used to treat tissue transplants to be validated for a specific SAL. At the same time, when an SAL approach is used, a manufacturer may have difficulty convincing national authorities that a

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4924 process with a "lower" SAL should be approved. There is a very important limitation of the SAL
4925 approach for sterilisation processes used for the treatment of tissue grafts: The SAL method cannot
4926 be used to quantify the efficacy of virus inactivation. For this reason, in countries with the most
4927 stringent quality standards for tissue grafts, an alternative approach is considered appropriate.
4928 b. Potency against a panel of bacteria, spores and viruses. Validation of potency can be achieved by
4929 application of the EN 1040 standard and/or Committee for Proprietary Medicinal Products
4930 (CPMP) guidelines [6, 7]. This type of validation requires that the maximum anticipated level of
4931 bacterial, fungal and viral contamination can be eliminated, by establishing the elimination
4932 capacity in terms of the number of log-scale reductions in the concentration of samples spiked
4933 with a panel of bacteria, fungi and viruses. The panel should cover Gram-positive and Gram-
4934 negative bacteria, spores, fungi and include known relevant "resistant" species. In the case of
4935 viruses, relevant species of enveloped and non-enveloped viruses covering the range of virus
4936 particle sizes should be included. Some authorities have published relevant guidelines [7].

4937 **8.6.2. Sterilisation methodologies applicable to tissue grafts**

4938 *8.6.2.1. Irradiation*

4939 Irradiation with gamma particles or accelerated electron beams can be used for the sterilisation
4940 and viral inactivation of tissue transplants. For the sterilisation component, such processes are well
4941 suited to validation according to SAL. Gamma radiation is effective in killing bacteria, fungi, spores
4942 and, to a more variable degree, viruses. However, depending on the dose and irradiation conditions,
4943 gamma radiation can have a negative effect on the mechanical properties of the grafts. Applying
4944 appropriate irradiation conditions can substantially reduce these negative effects and protect the graft
4945 integrity. There are data that suggest treatment of allografts with less than 25 kGy does not affect the
4946 integrity of the allograft [8]. Depending on bioburden, a ≥ 25 kGy irradiation dose may be required for
4947 sterilisation, and depending on the nature and extent of viral contamination a dose of ≥ 34 kGy may be
4948 required for virus inactivation [9].
4949 Such high doses and concomitant transient high temperatures (≥ 60 °C) are likely to have negative
4950 effects on transplant properties. It is, however, not possible to make generally applicable statements
4951 about the extent to which such effects will influence the clinical performance of transplants. The adverse
4952 effects of irradiation can be ameliorated by reducing the temperature and inclusion of radioprotectant
4953 chemicals [10, 11]. Irradiating musculoskeletal tissues in the frozen state retains the primary effects of
4954 gamma irradiation sterilisation (the breaking of covalent bonds by high-energy gamma rays) while
4955 minimising the secondary effects of the process (generation of free radicals). Overall, this can reduce
4956 the damage done to allografts by the irradiation process but may also provide some protection to micro-
4957 organisms.

4958 *8.6.2.2. Peracetic acid-ethanol treatment*

4959 This method is typical of the 'panel' approach in (b) above. In one example of a peracetic acid
4960 (1 %) ethanol sterilisation process, the method was tested in allogenic avital bone transplants with a
4961 thickness of 15 mm. The process led to a reduction of virus titres of more than 4 log10 TCID50/mL. For
4962 viable bacteria, fungi and spores, a titre reduction below the detection level (5 log10 cfu/mL) was
4963 achieved after an incubation time of 2 hours [12]. In the case of chemical treatment of tissues the question
4964 of potential residues could be a concern and the risk, if applicable, should be included in the "instructions
4965 for use".

4966 8.7. Requirements for processing facilities

4967 Facilities for aseptic and clean, non-sterile processing must be dedicated to this activity, and must be
4968 designed, qualified and monitored to ensure that the air quality is appropriate for the process being
4969 carried out (see Chapter 7).

8.7.1 Avoiding contamination and cross-contamination

In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during processing or storage, with tissues or cells from another donor, unless they are intentionally pooled. A separate set of clean, sterile instruments should be used for each donor. Where possible, these should be single-use and disposable. In some cases, e.g. for ocular tissue, single-use surgical instruments for procurement and processing are available and recommended. Each tissue or cell product should have a batch number that is also recorded in the processing records.

Pooled tissues or cells should be treated as a single batch, ensuring that full traceability to all the donations included in the pool is maintained. Due to the high probability of cross-contamination resulting from pooling, it must be supported by a thorough risk–benefit analysis.

8.8. Quality control

8.8.1. General considerations

Tests and procedures should be carried out to measure, assay or monitor processing, preservation and storage methods, equipment and reagents to ensure compliance with established tolerance limits. Written procedures must be in place that govern quality control at key stages during processing. The written procedures should include as a minimum the test method, the sample size and the acceptance criteria. The minimum requirements for evaluation of each type of tissue and cell are described in tissue- and cell-specific chapters (see Chapters 17-35). The results of all tests or procedures should become part of the permanent processing record.

If in-process controls are undertaken in the processing area, they should be carried out so that there is no risk to the processing steps being followed.

8.8.2. Microbiological testing

In many cases, it is not possible to exclude contaminated material during processing because the tissue originates from parts of the body which contain natural microbial flora, and pre-processing disinfection is not 100 % effective. The microbiological safety of tissues and cells is based on donor selection and minimisation of initial contamination, with protocols to control and monitor contamination being employed during the entire procurement process. Chapter 10 describes methods of microbiological control. Sampling and testing methods must be validated to demonstrate that the sampling method accurately represents the tissue, and that the testing methods are suitable and fit for purpose.

Various procedures exist for securing microbiological control, such as decontamination by antibiotics, or physicochemical methods. If physicochemical methods are to be applied, these procedures must be adapted to the type of tissue or cell and should be validated. The effectiveness of a decontamination or inactivation procedure should be shown for relevant micro-organisms in the tissue or cell preparation itself and not only in an aqueous solution. The risk that some micro-organisms may survive decontamination with antibiotics, but not be detected by post-decontamination microbiological testing, must be considered. This factor has been implicated in the death of, and serious injury to, patients [13, 14].

Non-conforming products must be identified and separated from conforming products. The fate of non-conforming products will be decided by the Responsible Person (RP) in charge of the TE.

8.9. Significant changes

A documented risk assessment approved by the RP must determine the fate of all stored tissues and cells following the introduction of any new donor selection or testing criterion, or any significantly modified processing step, that enhances safety or quality. Guidance on risk assessment is provided in Chapter 2.

A documented change-control procedure must be followed before any significant change is implemented in processing. This must be supported by a comprehensive risk assessment. The purpose

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5015 of this is to ensure that the change is justified, is documented and will not affect the quality of the product
5016 (see Chapter 2). All relevant persons should be involved in evaluation of the change.
5017 As a minimum, the following aspects of any change in processing should be evaluated:
5018 a. significance;
5019 b. effect on quality;
5020 c. need to update SOPs;
5021 d. need to re-validate the process;
5022 e. effects on quality-control (QC) analyses;
5023 f. need to inform regulatory authorities;
5024 g. need to train personnel;
5025 h. effect on risk analyses.

5026 8.10. Process validation

5027 If processing is carried out according to GMP, the processing validation must also be done according to
5028 GMP guidelines. In the EU, Commission Directive 2006/86/EC allows for validation studies to be based
5029 on any of the following:
5030 a. studies undertaken by the establishment itself;
5031 b. data from published studies;
5032 c. for well-established processing procedures, retrospective evaluation of the clinical results for
5033 tissues and cells supplied by the establishment.

5034 Where validation is based on studies carried out by the establishment itself, reports should include
5035 at least the following elements:
5036 a. a validation plan that specifies the critical parameters to be assessed and the acceptable result
5037 thresholds for these parameters;
5038 b. a documented methodology;
5039 c. all results obtained, described clearly and with relevant interpretation;
5040 d. a signed declaration of validation acceptance or rejection by the quality manager (QM) or the RP.

5041 Validation studies should be carried out by applying 'worst case' scenarios. The equipment used
5042 for validation studies should be fully qualified, and measuring devices should be calibrated to traceable
5043 standards. Validation experiments should be repeated at least in triplicate, though this will depend on
5044 the degree of variability in the data, to ensure reliably repeatable results. For an example of a validation
5045 study, see Appendix 4 (Example of cleanroom qualification), Appendix 5 (Example of incubator
5046 qualification), Appendix 6 (Example of process validation) and Appendix 7 (Example of method
5047 validation (oocyte vitrification) in assisted reproductive technology).
5048 Where validation is based on data from published studies, the relevant publications should be
5049 filed as part of the validation record. In this case, the TE should demonstrate that they can effectively
5050 reproduce the published process with the same results in their facility (operational validation). Copies
5051 of the relevant SOP and the results of the operational validation should be provided, to demonstrate that
5052 the process is equivalent to that applied in the scientific literature.
5053 Where specific steps have been significantly modified or adapted, separate validation should
5054 confirm that these changes have not invalidated the method. There should be a signed declaration of
5055 validation acceptance or rejection by the QM or RP.
5056 If validation is based on retrospective evaluation of the clinical results for tissues or cells supplied
5057 by the establishment (i.e. for well-established processing procedures), data should be collected and
5058 analysed that include the number of tissues or cells implanted following processing by the method under
5059 consideration, and the time period (start and end dates/times) during which these implantations occurred.
5060 It should be demonstrated that, where a vigilance system was already in place at the time, clinical users
5061 were informed of the procedure for reporting adverse reactions. There should be a signed declaration of
5062 validation acceptance or rejection by the QM or RP.

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The procedures used to prevent or reduce contamination during processing may vary, depending on the type of tissue and how it is processed. However, they should all be fully validated. Decontamination methods, such as antibiotic soaking, should be validated to demonstrate effectiveness against a range of contaminants similar to those routinely found on the tissues or cells in question. Such studies should be designed to ensure that residual decontaminants (e.g. antibiotics) do not affect the validity of the microbial tests carried out on the product.

If the process includes a sterilisation or viral-inactivation step, process-specific validation studies should be completed to demonstrate the log reduction achieved by the process.

Subsequent to process validation and during routine processing, TEs should monitor tissue and cell quality to ensure a state of quality control is maintained throughout the processing part of the product lifecycle. This will provide assurance of the continued capability of the process and its quality controls to produce finished tissues and cells that meet the desired quality and to identify changes that may improve product quality or performance. Relevant process trends (e.g. quality of incoming materials or components, in-process and finished product results, cases of non-conformance and defect reporting) should be collected and assessed to verify the validity of the original process validation or to identify required changes to the associated controls. Documentation and tracking of patient outcomes constitute a critical element of ongoing process verification. For new or significantly changed processes, a system to enable close clinical outcome monitoring should be agreed with clinical users.

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Related documents:

Appendix 4. Example of cleanroom qualification

Appendix 5. Example of incubator qualification

Appendix 6. Example of process validation

Appendix 7. Example of method validation- oocyte vitrification.

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5118 # Chapter 9. Storage and release

5119 ## 9.1. Introduction

5120 Storage is the maintenance of tissues and cells for clinical application under appropriate controlled
5121 conditions until distribution, and it occurs at various stages from procurement to clinical use. Storage
5122 must be controlled and documented to ensure that the required properties of the tissues or cells are also
5123 maintained during storage and that cross-contamination or loss of functionality, efficacy, clinical
5124 effectiveness and traceability is avoided.

5125 The opportunity to store tissues and cells is also common during the process and brings great
5126 advantages:

5127 a. preservation of the required properties of the biological material, making extended storage for
5128 future use possible;
5129 b. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of
5130 tissues or cells;
5131 c. reducing the risk of disease transmission by testing of infectious diseases and microbial
5132 contamination prior to release and clinical use, though this is only possible if the tissues and cells
5133 can be stored under defined conditions for an adequate time in order to await the test results;
5134 d. performing and awaiting other quality-control results before release (e.g. cell counts, clonogenic
5135 assays for hematopoietic progenitor cells (HPC), residual moisture in lyophilised or dehydrated
5136 grafts).

5137 Each tissue establishment (TE) must have a documented policy on tissue and cells release, and
5138 specifications against which tissues and cells are verified. These specifications must include the
5139 technical requirements and other criteria considered by the TE to be essential for the maintenance of
5140 acceptable quality. In general, release is the final evaluation and control that these specifications of
5141 tissues or cells are met. Only then, the tissues or cells can be distributed to the hospital and used for
5142 clinical application.

5143 This chapter provides generic guidance on the storage and release of tissues and cells carried out
5144 by TEs. Further tissue and cells specific guidance is provided in Part B of this Guide.

5145 ## 9.2. Storage

5146 ### 9.2.1. General

5147 Storage facilities for materials, tissues and cells are present in most TEs, procurement
5148 organisations, donation and transplant hospitals, organisations for human applications and pharmacies.
5149 These storage facilities must have policies and SOPs for all processes that affect quality and safety.

5150 Storage facilities must ensure that the equipment being used, the working environment, the
5151 process design and the qualification, validation and control conditions are in compliance with
5152 established quality and safety requirements (see Chapter 2). Storage (during the process and after
5153 release) must be carried out as defined in the specifications for the specific tissues or cells to guarantee
5154 the quality and safety of tissues and cells. Organisations must have a dedicated area or material for
5155 quarantine (temporary storage) and a plan for back-up storage. An inventory of tissues and cells stored
5156 must be performed regularly.

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If an organisation entrusts one of the stages of storage to a third party, a written agreement is needed between the TE and the third party. The TE must evaluate and select third parties based on their ability to meet the established standards of quality.

The recommended time limits between procurement, processing and storage are described in the tissue- and cell-specific sections of this Guide (see Part B). Where appropriate, these maximum times from procurement (or cardiac arrest) until processing and storage must be defined to maintain quality, safety and clinical effectiveness of the tissues and cells. Procurement, processing and storage times must be documented in the records for tissues and cells.

Reference samples of tissues and cells for quality control should be stored under the same conditions as the tissues and cells themselves (e.g. HPC and cord blood reference samples).

9.2.2. Methods of storage

Following processing, tissues and cells should be stored according to currently accepted good practice, based on the best available scientific evidence and according to good manufacturing practice (GMP), as appropriate, for tissues and cells. All procedures associated with storage of tissues and cells must be documented in SOPs.

9.2.3. Storage temperature

Refrigeration devices/incubators containing tissues and cells should be suitable for the intended use, and the procedures for monitoring such devices should be appropriate so that tissues and cells are maintained at the required storage temperature. Regular monitoring and recording of temperature, together with suitable alarm systems, must be employed on all incubators, storage refrigerators, freezers and liquid nitrogen tanks (see Chapter 2). The functionality of the alarm systems must be checked regularly. Temperature ranges for storage of tissues and cells are shown in Table 9.1.

Table 9.1. Temperature range for storage of tissues and cells

Storage condition	Temperature range (°C)
Cryopreservation	< −140
Deep frozen [a]	−80 to −60
Frozen [b]	< −15
Refrigerated [b]	2 to 8
Cold or cooled [b]	8 to 15
Room temperature [b]	15 to 25
Organ culture	28 to 37

a. Based on general practice.
b. Based on the *European Pharmacopoeia* [1].

9.2.4. Requirements for storage facilities

Facilities for storage must be dedicated to this activity, and must be designed, qualified, validated and monitored to ensure appropriate conditions (see §7.2).

Although storage facilities do not need to strictly follow the same environmental criteria as the procurement and processing facilities, once the tissues and cells have been processed and stored/banked it is wise to invest in resources to maintain a secure, clean and stable environment for long-term storage.

The storage room must have a sufficient area in an appropriate place and must be designated for the specific purpose for which it is used; therefore, it must have enough space to allocate the number of tanks, refrigerators and/or freezers to store the expected number of samples. There must also be sufficient space for the movement of equipment, samples and personal. It is recommended that the room for manoeuvre is at least the size of the largest equipment or container. Furthermore, the access to the

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5191 storage room must be through a door whose opening is larger than the diameter of the largest equipment
5192 in the room.
5193 The room should be in a dry, cool, well-ventilated place, free from heat sources.
5194 For security reasons access to the storage facilities should be restricted to authorised personnel.
5195 Appropriate alarms for temperature control and low levels of oxygen must be put in place, along with
5196 sensor systems in case of liquid nitrogen leaks. In case of loss of electrical power, as a part of the TE
5197 general emergency plan, the storage facility should have generators or uninterrupted power supply
5198 (UPS) systems.

5199 **9.2.5. Selecting appropriate air quality for storage**
5200 In order to avoid heavy environmental microbial contamination, certain areas such as corridors
5201 or open access from outdoors should be avoided. Storage rooms can be equipped with an HVAC
5202 (heating, ventilation and air conditioning) system for maintaining the temperature and humidity and
5203 adequate air exchange (e.g. to prevent accumulation of nitrogen; see §9.2.7).

5204 **9.2.6. Environmental monitoring**
5205 Similarly, as with the processing rooms, if storage is carried out according to GMP and defined
5206 in the in-house environmental hygiene programme, monitoring systems for storage rooms may need to
5207 be put in place. The frequency of $>0.5\,\mu m$ particle monitoring, as well as the implementation of alarm
5208 limits for particle concentration, will depend on the degree of stringency needed and the principles of
5209 quality risk management. Together with environmental airborne particle monitoring, microbiological
5210 contact-plate monitoring may be performed to demonstrate the degree of cleaning of the room (see
5211 §7.5.3 and §7.5.4).

5212 **9.2.7. Special safety measures for liquid nitrogen facilities**
5213 The organisation of the room must allow circulation and manipulation around the cryogenic tanks.
5214 The room must be clearly identified with pictograms indicating the dangers and the presence of personal
5215 protection equipment. The staff need to be specially trained and qualified for these working conditions.
5216 Before entering the room, the staff must be able to ensure that ventilation is functioning correctly
5217 and be able to check the oxygen level. An adapted mechanical ventilation (extraction and fresh-air
5218 intake) system, working continuously, is mandatory, ensuring renewal of the air. Continuous control of
5219 the oxygen rate is ensured by several detectors, checked at least once a year. They are put in the lower
5220 areas of the room where the ventilation is at its weakest. The oxygen rate or alarm status must also be
5221 visible outside the room.
5222 Following nitrogen evaporation, leading to an oxygen level equal to or lower than 19 %, an alarm
5223 (visual and audible) is activated and staff must then leave the room and await normalisation of the
5224 oxygen level. The alarm is connected to a continuous monitoring station allowing alerts for the staff
5225 working in the room and the rescue or assistance staff, if necessary.
5226 Tank-filling systems should be designed to minimise evaporation of nitrogen. They should be
5227 equipped with safety valves and rupture discs, and external degassing valves for long lines.
5228 The temperature of the room is 21°C ±3 °C to prevent condensation on the tanks and the formation
5229 of ice inside them. The floor is covered with a material resistant to low temperatures and high loads,
5230 allowing easy movement of the tanks without shock. The tanks are to be maintained and checked
5231 annually.
5232 The entrance door of the room is equipped with an oculus allowing surveillance from outside.
5233 The opening of the door is outwards. Gloves with long sleeves able to protect against cold, with non-
5234 combustible capacities, and safety glasses (EN166) or visors protecting the face are to be made available
5235 to the staff. (For further details, see §7.2).

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9.2.8. Avoiding contamination and cross-contamination

Storage conditions must prevent mix-ups, contamination and cross-contamination of tissues, cells, supplies and reagents (see also §7.6) [2, 3, 4]. Areas designated for storage of cells and tissues in process, under quarantine, and released for distribution must be established and controlled. In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during storage, with tissues or cells from another donor, unless they are pooled intentionally. Every effort should be made to avoid cross-contamination of material. Tissues and cells immersed in liquid nitrogen should be double-wrapped during storage (depending on the storage system, type of sample and after risk assessment). This may not be mandatory for reproductive cells; see §27.6.8, Processing of samples from seropositive donors in partner donations.

This is highly important for storage with liquid nitrogen owing to the accumulation of microbial contaminants in liquid nitrogen storage vessels, as well as to avoid cross-contamination among samples. The seals and the material employed must be validated for their use at the designated storage temperature and for the conditions of use, to demonstrate that the packaging and labelling can retain their integrity under such conditions.

In principle, to avoid contamination of tissues and cells, also the entry of personnel to the storage facilities should be reduced to a minimum.

Additionally for periodical cleaning, a cleaning plan for the sanitation of the storage equipment should be implemented, depending on the type of equipment. During the cleaning process, a back-up unit must be used to provide the same safe conditions as the storage equipment. The emptying for cleaning and maintenance should be scheduled in advance and needs to be performed following an SOP.

9.2.9. Types of storage

9.2.9.1. Quarantine storage

All human tissues and cells that are stored before having determined their suitability must be kept under quarantine. Quarantined tissues and cells should be physically separated and visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from released tissues. An SOP must describe how to categorise quarantined and released tissues and cells.

Specific areas must be defined for tissues and cells in quarantine and for tissues and cells that have been released; a separate area must be dedicated to the storage of medium and other materials used during the process of preparation. Access to storage facilities must be restricted to authorised persons. If the storage devices are located in a shared facility with other users, they must be securely locked.

9.2.9.2. Short-term storage

Because of processing methods and the life span of cells, some viable tissues and cells can only be stored for a short period of time (e.g. cornea for about 4-5 weeks).

9.2.9.3. Long-term storage

If cell viability should be maintained for a longer period, other strategies such as cryopreservation or lyophilisation need to be considered.

Obtaining and analysing the critical clinical outcome data can provide evidence to be used to verify the safety and efficacy of the storage procedures.

9.2.9.4. Storage at an organisation responsible for human application (ORHA)

ORHAs have short- and long-term-storage for tissues awaiting inspection, surplus or unused tissues and tissues recalled by TEs. Therefore, a system for identification of tissues and cells throughout any phase of the clinical application at the ORHA must clearly distinguish tissues and cells released from quarantine and/or discarded ones (see Chapter 12).

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9.2.10. Expiry date

To ensure the maximum safety and quality of tissues and cells, it is mandatory to specify a maximum storage time with an expiry date for each type of storage condition. The chosen maximum storage period should be validated, based on data from published studies, stability testing by the establishment or evidence-based facts (e.g. retrospective evaluation of the clinical results for tissues and cells supplied by the establishment). When determining the maximum storage period, several factors should be considered. These include (but are not limited to):

a. possible deterioration of the required properties of tissues and cells;
b. risks related to microbiology;
c. tissue availability;
d. package integrity over time;
e. expiry of storage solutions;
f. stability at the storage temperature;
g. overall risk assessment of quality assurance: donor evaluation, donor testing (kits), quality criteria (viability, functionality after thawing), regulations.

When relevant for the type of tissue or cell, the time of procurement should also be indicated. In certain specific cases, it may be possible to prolong the expiry date, especially in the case of cryopreserved HPC (i.e. cord blood), reproductive tissues and cells for partner donation or in cases of fertility preservation (see chapters 27 and 28), e.g. MAR/ART do not have expiry dates, and HPC are retested before release, even after decades of storage.

9.2.11. Storage validation

If storage is carried out according to GMP, the storage validation must be done according to GMP guidelines. Further details can be found in Chapter 2 on validation.

Regarding storage validation, homogeneity and reproducibility are fundamental requirements of tissues and cells storage/banking. No matter which type of storage strategy is used, it will be fundamental to demonstrate that tissues and cells stored will be able to achieve a successful clinical outcome. Stored material should be checked to ensure that both maintenance and documentation are updated and that any changes have been accordingly recorded.

Storage design should prevent accidental release and distribution of tissues or cells.

In case of dissolution of the establishment, facilities must have third-party agreements for transferring stored usable tissues and cells to another storage facility or for disposing of the unusable tissues and cells.

9.3. Release

9.3.1. Release procedure

Release is the act of certifying compliance of a specific tissue or cells or batch of tissues or cells with the requirements and specifications. Before any tissues and cells are released, all relevant records (including donor records, processing and storage records, and post-processing quality-control test results) must have been reviewed, approved and documented as acceptable by an authorised and trained person according to the relevant local SOP and national regulations. For those countries that are members of the EU, the records must be checked by the Responsible Person (RP) meeting qualification criteria defined in Directive 2004/23/EC or by a person authorised for this task by the RP. There must be an SOP that details the specifications, circumstances, responsibilities and procedures for the release of tissues and cells.

At the time of release, donor records and tissue- or cell-processing records should be reviewed to ensure that the material is suitable for clinical use and implantation. The review should include:

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5325 a. pproval of donor eligibility by the RP or designated person;
5326 b. review and approval of the processing and storage record (including environmental
5327 monitoring records);
5328 c. final evaluation of the label and container to ensure traceability, accuracy and integrity (these
5329 tasks can be delegated);
5330 d. results of screening tests on incoming material and in-process controls;
5331 e. specifications for final release of tissues or cells based on testing results used to determine
5332 final release (e.g. quality controls such as. viability or cells count, microbiology test results;
5333 if necessary and justified, the final release can be undertaken on a 'negative-to-date' basis);
5334 f. absence of any serious adverse reactions and events (from previous or same donation) over
5335 the time period; in cases of MAR, the final clinical users must report the clinical outcome
5336 even in the absence of any SAE or SAR;
5337 g. in cases of MAR/ART (gametes and embryo donation), check that the total of permitted live
5338 births does not exceed the national regulations;
5339 h. if the tissue or cells are used autologously, the same review should be performed; if there
5340 are positive test results, it is up to the decision of the RP and the treating doctor whether the
5341 tissue or cells can be used, but in any case the final decision has to be justified and recorded.

5342 The items indicated in the release record should contain at least:
5343 a. the procurement file and/or release statement of the person responsible for procurement;
5344 b. type(s) of tissues and cells processed and/or stored (number of units per device or ampoule);
5345 c. quantitative and qualitative description of the tissues and cells processed, preserved and/or
5346 stored;
5347 d. date and time of each stage of processing and storage, identification of persons responsible
5348 for each step and the identifying reagents and materials used (batch number and expiry date);
5349 e. status of tissues and cells at all stages of processing and storage (i.e. quarantine, release for
5350 therapeutic use, release for manufacture of medicinal products, *in vitro* research, etc.);
5351 f. use of antibiotics, antibiotic composition and incubation period (if applicable);
5352 g. type and amount of reagents used;
5353 h. procedures and records concerning the processing of tissues and cells (if applicable);
5354 i. processing data (preparation, culture technique, incubation, treatment chemicals,
5355 confirmation of clean room status);
5356 j. data on techniques of decontamination, sterilisation or viral inactivation;
5357 k. results of specific quality testing, depending on tissue and cell type (e.g. human leucocyte
5358 antigen (HLA), histology, radiology results, tissue or cell viability, number of CD34 cells);
5359 l. procedures and records concerning the preservation of tissues and cells (e.g.
5360 cryopreservation, trace of the cooling curve, glycerolisation, lyophilisation), if applicable;
5361 m. date and time of storage;
5362 n. method of storage;
5363 o. storage temperature;
5364 p. expiry date, if applicable;
5365 q. identification of tissues and cells (i.e. donor identification code and product code). Further
5366 information on coding, labelling and packaging can be found in Chapter 14.

5367 Access to registers and recorded release data must be restricted to authorised persons. These
5368 records must be kept for a minimum of 30 years after clinical use or discard of tissues and cells.
5369 The person responsible for the release of tissues or cells (RP) should sign a statement that specifies
5370 fulfilment of all legal requirements, tissue and cells specifications and quality-release criteria as defined
5371 by the TE, thereby releasing the tissues and cells for storage in an inventory of tissues and cells that are
5372 available for human application. If release can not be approved, the tissues or cells need to be discarded

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or can be made available for research/educational use, if a specific consent for research/educational use was given.

Released tissues should be physically separated and made visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from quarantined tissues. The TE must provide clinical users with instructions for using the tissue or cells. Clinical users must be reminded that they must report any adverse events or reactions to the TE and are responsible for traceability to the recipient (see Chapter 15).

If the tissues or cells do not comply with all specifications, there can be an exceptional release under some conditions (see §9.3.2). If autologous tissue is released with positive test results (e.g. infectious disease marker, microbiological controls), the clinical user must be informed in written form before the tissue is distributed to the hospital.

9.3.2. Exceptional release

In exceptional circumstances, a TE may agree with the organisation responsible for human application of tissues and cells (ORHA) and the treating doctor that tissues or cells that do not meet the normal criteria for release can be released and used in a specific patient, based on a risk–benefit analysis taking into consideration the alternative options for the patient and the consequences of not providing the tissues or cells. For more information, see Chapter 12.

9.3.3. Risk assessment

A documented risk assessment approved by the RP must determine the fate of all stored tissues and cells following the introduction of any new donor-selection or testing criterion or any significantly modified processing step that enhances safety or quality. Guidance on risk assessment is provided in Chapter 2.

9.3.4. Disposal of human tissues and cells

There must be a documented policy for disposal of tissues and cells that are unsuitable for clinical use. Records should include details of date, involved personnel, method of and reasons for disposal. The material should be handled appropriately and disposed of in a manner compliant with local control of infection guidelines. Human tissues, cells and other hazardous waste items should be disposed of in such a manner as to minimise the hazards to the TE's personnel or the environment, and should be in conformity with applicable European, national and local regulations.

Disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and the human body. For HPC and autologous tissues and cells it is necessary to document that the conditions for disposal defined in the consent prior to collection have been met, including (where applicable) the option to transfer the tissues and cells to another facility if the designated recipient is still alive after the agreed storage period.

In case of MAR/ART, the donors and patients must declare in writing the destiny of their reproductive material when the maximum storage period has ended (see Chapter 27). A specific signed consent is needed for the acceptance of the final disposal of a given sample.

Disposal of cellular therapy products shall include a pre-collection written agreement between the storage facility and the designated recipient or the donor, defining the length of storage and the circumstances for disposal of cellular therapy products [5].

9.4. References

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Chapter 10. Principles of microbiological testing

10.1. Introduction

This chapter addresses the control and examination of microbiological, endotoxin and mycoplasma contamination of human tissues and cells, a process which is critical in ensuring the quality and safety of human tissue and cell grafts used for human application. This chapter also defines the approaches to, and requirements for, effective and meaningful microbiological testing of preparations of tissues and cells and the environments in which they are processed. It describes general principles that should be adopted in developing a comprehensive strategy for microbiological testing, which are based on the use of the *European Pharmacopoeia* (*Ph. Eur.*) microbiological test methods. However, specific characteristics of human tissues and cells must be considered and methods adapted accordingly. Guidance on microbiological testing for particular tissue and cell processes is provided in tissue- and cell-specific chapters.

All facilities that procure, process or store tissues and cells should have access to the services of a microbiology laboratory with a fully implemented quality-management system and access to the advice of a suitably qualified expert microbiologist.

10.2. Microbiological examination of donors

Microbiological blood cultures can be a useful tool for the diagnosis of bacteraemia and other infections in deceased donors [1] and living donors (e.g. stem-cell donors) of tissues and cells, in addition to the required serological examinations. The blood samples must be of sufficient quantity, collected properly and accompanied by relevant clinical information. Compliance with aseptic techniques has to be assured in order to evaluate the origin of a detected contamination properly.

For blood cultures, a clear protocol should be followed that addresses skin disinfection, the amount of blood obtained and the number of blood cultures. Skin disinfection is an important prerequisite for reliable results to avoid secondary contamination of the sample. At least a two-step alcoholic disinfection (preferably accompanied by sporicidal disinfection) should be done. Larger quantities of blood as well as several independent blood cultures improve the probability of detecting a bacteraemia in the donor. It is recommended to take at least 2-4 blood cultures (each aerobic and anaerobic), ideally at different time points and from different vessels. The blood cultures have to be incubated for ≥ 5 days.

- Blood samples for culture can be obtained before or after circulatory arrest. The results of blood cultures using samples collected before circulatory arrest provide useful information about the clinical status of the donor (e.g. infections with objectionable organisms) and can be a useful supplementary tool for evaluation of donor suitability. Blood cultures are valuable in evaluating the quality and safety of specific tissues and cells especially if the tissues and cells are not terminally sterilised. The evaluation depends on numerous factors and should be based on knowledge of the particular settings, e.g. time points of blood withdrawal [2-8].
- In the situation where blood cultures are obtained sometime after cardio-circulatory arrest (in which organs, tissues and cells may be at a higher risk of endogenous microbiological contamination) the information provided by blood cultures may be questionable because the course of *post mortem* distribution of micro-organisms is (to a large extent) unknown, and the results can be influenced by agonal spread and *post mortem* bacterial translocation. The conditions under which the deceased donors are stored may be highly variable. On the other

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5467 hand, information about *post mortem* bacteraemia and fungaemia at the time of procurement
5468 may also be useful to assess the quality and suitability of tissues and cells.

5469 The main objectives of *post mortem* cultures in the context of tissue and cell donation are three:
5470 i. to record infections that could be either clinically unsuspected or clinically suspected but
5471 not proven *ante mortem*,
5472 ii. to evaluate the efficacy of antimicrobial treatment in the case of procurement of tissues,
5473 and
5474 iii. to check contamination from the procurement team and environment as well as cross-
5475 contamination between tissues or cells preparations.

5476 The theories supportive of the ambiguity of *post mortem* culture results are agonal spread and
5477 *post mortem* bacterial translocation. Although agonal spread is less common than assumed and
5478 prevention is difficult, it appears that *post mortem* bacterial translocation is completely overcome if the
5479 body is refrigerated soon after death [2, 3]. Manipulation of the deceased donor can lead to dissemination
5480 of micro-organisms from the lung and visceral organs to the heart. However, published data suggest that
5481 neither agonal nor *post mortem* spread can be expected to produce false-positive cultures if the body is
5482 appropriately cooled, if the tissue procurement is performed within 24 hours after death and if the
5483 samples for microbiological culture are collected early during tissue procurement, with minimum
5484 possible manipulation of the deceased donor before manipulation of the gastro-intestinal tract [2, 3].
5485 Nevertheless, a positive culture, in the particular context of organ, tissue or cell procurement, may
5486 signify contamination or cross-contamination during procedures, indicating problems with *post mortem*
5487 microbial spread, recovery techniques and/or environment. These possibilities should be evaluated
5488 because they may affect the suitability of particular tissue and cell preparations and of other tissues or
5489 cells that eventually could have been affected by cross-contamination, or may imply that particular
5490 attention should be applied to decontamination and/or sterilisation methods.

5491 ## 10.3. General considerations for microbiological testing

5492 The approaches outlined in this section cover the microbiological testing of procurement, processing,
5493 storage and release of tissues and cells. Microbiological testing by sampling and culturing of the tissues
5494 or cells is the most direct measure of microbiological contamination.

5495 ### 10.3.1. Sampling

5496 In principle, microbiological testing must be carried out on representative pre-processing samples
5497 of the procured tissues or cells and on post-processing samples of every final tissue and cell graft, since
5498 a number of microbial contaminants can firmly adhere to or persist inside tissues and cells [9]. Sampling
5499 must be completed immediately before packaging or as late as possible during the procurement or
5500 manufacturing process. In cases where the nature of the procured tissues and cells or final tissue and cell
5501 grafts does not allow sampling, an alternative sampling approach may be undertaken. For example,
5502 transport, storage, rinsing, washing or culture solutions can be used instead of the primary samples. If
5503 an appropriately validated terminal sterilisation process is applied, testing of samples within a batch has
5504 to be undertaken to monitor the effectiveness of the terminal sterilisation process.
5505 In addition, where applicable, in-processing samples should be tested on a regular basis according
5506 to a risk assessment, considering the nature of the tissues and cells and critical processing and storage
5507 steps. In-process testing should be performed at relevant steps of the production process, such as:
5508 • bioburden testing before a terminal sterilisation procedure or decontamination procedure (e.g.
5509 antibiotic soaking);
5510 • microbiological testing after a stage of decontamination or inactivation, before final storage,
5511 though ideally a disinfectant or antibiotic-free period should precede sampling;

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5512 • microbiological testing after washing steps or changing of the storage medium, particularly
5513 when decontamination processes cannot be applied.

5514 **10.3.2. Testing for bacteria and fungi**

5515 Aerobic, anaerobic and fungal testing of tissue or cells samples under appropriate incubation
5516 conditions is the most direct qualitative measure of microbiological contamination and must be
5517 conducted for pre-processing samples of the procured tissues and cells and on post-processing samples
5518 of every final tissue and cell graft. Considering the nature of the procured tissues and cells and any
5519 subsequent processing steps, the microbiological testing approach should follow the procedures outlined
5520 in the *Ph. Eur.* in particular chapters 2.6.1, 2.6.12, 2.6.13 and 2.6.27. Deviations from such standards
5521 should be justified, and alternative test methods must be validated in accordance with chapter 5.1.6 of
5522 *Ph. Eur.* Factors such as samples containing antibiotics or very small sample amounts may affect the
5523 sensitivity of tests leading, in the worst-case scenario, to false-negative results. Testing should be
5524 extended to specific micro-organisms known to represent potential contaminants on the respective
5525 tissues or cells where transmission may become relevant for infection [10]. These micro-organisms may
5526 not be detectable with common culturing media; therefore, additional tests for specific infectious agents
5527 should be undertaken in compliance with *Ph. Eur*. methods. If applicable, an exclusion list for non-
5528 acceptable micro-organisms should be compiled.
5529 If release of the tissues or cells is necessary before the end of the officially verified/required
5530 testing period, negative-to-date reading of the results may be carried out. In this case, intermediate
5531 results of the final testing in combination with intermediate or final results of in-process testing are used
5532 for tissue or cell release. The clinician using the relevant graft must be notified so they can decide if its
5533 application is clinically justified. Final testing is still ongoing after the tissue or cell graft is released and
5534 will be completed in line with the above-mentioned requirements. If micro-organisms are detected after
5535 tissue or cell release, predefined measures such as identification and antibiotic sensitivity of the species
5536 must be carried out and information must be provided immediately to clinicians caring for the patient.
5537 Alternative, rapid microbiological methods should be considered, especially for preparations of tissues
5538 and cells with a short shelf-life. Independent of the applied method, their validity must be shown
5539 according to *Ph. Eur.* 2.6.27 or 5.1.6. Deviations from these standards should be justified.

5540 **10.3.3. Testing for mycoplasma**

5541 Depending on the type of preparation and manufacturing process, it can be necessary to
5542 complement the microbial test concept by additional tests for specific infectious agents such as
5543 mycoplasma (*Ph. Eur.* 2.6.7). To identify and assess the contamination risk of specific tissues or cells, a
5544 sufficient number of samples from different tissue or cell batches should be examined. Possible sources
5545 for mycoplasma contamination include the cellular starting material itself or its procurement, animal- or
5546 human-derived raw materials such as untreated sera, personnel in the clinic or manufacturing site, and
5547 the entire manufacturing process.
5548 Mycoplasma can penetrate sterilising-grade filter membranes with a nominal pore size of
5549 $\leq 0.2\,\mu m$. They also lack a rigid cell wall, which makes them unsusceptible to antimicrobial agents that
5550 target the cell wall. Furthermore, many broad-spectrum antibiotics inhibit the proliferation of
5551 mycoplasma but do not kill them. Hence, elimination with antibiotics is difficult.
5552 Contamination with mycoplasma represents a potential risk for the patient because of transfer of
5553 infectious microbial agents into a potentially immunocompromised patient, but the effect of
5554 mycoplasma contamination on the tissue or cell preparation might also be critical. Mycoplasma are
5555 known to alter cell function, leading to an alteration of gene expression, cellular signalling and metabolic
5556 activity.
5557 Testing should be conducted at manufacturing steps at which mycoplasma contaminations would
5558 most likely be detected, such as after pooling or collection but before washing steps. Mycoplasma are

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5559 cell-associated micro-organisms that may even locate within the cell, so testing should always include
5560 the cellular matrix, if possible.

5561 **10.3.4. Testing for bacterial endotoxins**

5562 The need for routine testing for endotoxins is dependent upon the intended application of the
5563 tissues and cells, and the estimated impact of endotoxins on the recipient. For example, endotoxins in
5564 pancreatic islets will negatively affect insulin production and the outcome of transplantation. If deemed
5565 necessary, endotoxin testing should be carried out according to *Ph. Eur.* 2.6.14. In any procedure in which
5566 animal products are used (e.g. collagenase, trypsine), endotoxin testing should be done.

5567 The presence of endotoxins in tissues or cells can result in responses ranging from fever to
5568 irreversible and fatal septic shock. Endotoxins are the lipid portions of the lipopolysaccharide (LPS)
5569 macromolecule structures of Gram-negative bacteria, and of some cyanobacteria, which form an integral
5570 part of the cell wall. During bacterial growth, membrane complexes containing endotoxins may exist,
5571 bound to the cell surface or shed in small amounts into the environment, but they are released in greater
5572 concentrations on the death and lysis of the cell. These complexes accumulate both *in vitro* and *in vivo*,
5573 and release is enhanced by cell lytic compounds and antimicrobial agents.

5574 Endotoxins have important roles in cell cultures because they can alter the evolution of cell
5575 cultures and thereby impair the safety and efficacy of the cell graft. The potential sources of endotoxins
5576 in cell cultures are:

5577 a. glassware and plastic ware used in the laboratory;
5578 b. washing solutions or water used to prepare media and solutions;
5579 c. media and sera used during cell culture;
5580 d. any components and additives.

5581 Hence, it is recommended to use raw materials certified to be free of endotoxins by their
5582 manufacturers and to utilise pre-sterile, single-use items whenever practicable.

5583 Each laboratory that works with cell cultures should have a specific risk assessment and risk
5584 analysis that should include when and how to carry out an endotoxin test, together with all
5585 microbiological controls that are considered necessary. It is recommended to carry out an endotoxin test
5586 on the final cell graft before release to the patient.

5587 Several methods have been applied for the determination of endotoxin levels in a sample. Today
5588 the most widely used assay method for endotoxin is the Limulus Amoebocyte Lysate (LAL) assay based
5589 on the reaction of LPS with a clotting protein secreted by the horseshoe crab *Limulus polyphemus*. There
5590 are three basic LAL methods: the gel clot (kinetic method), turbidity measurement and chromogenic
5591 assay. The latter two are kinetic methods and are claimed to be more sensitive, being able to detect
5592 0.001 endotoxin units/mL [11]. Several factors – such as pH, protein concentration, metal ions and some
5593 chemicals – can influence the sensitivity of LAL tests. Different endotoxins may differ markedly in their
5594 activity by weight in both pyrogen testing and LPS-specific methods; the reference standard may also
5595 vary between laboratories, making comparisons difficult. Consequently, endotoxin activity is expressed
5596 universally as endotoxin units (EU) against a LPS standard preparation of a reference strain of *E. coli*.
5597 As a guide, 1 EU is considered to represent approximately 0.1 ng (10^5 fg) of endotoxin and is equivalent
5598 to a weight between 2 and 50 fg/cell or between 2,000 and 50,000 bacterial cells, depending on the
5599 bacterial species.

5600 In terms of a risk threshold, the maximum permissible level of endotoxin in injectable cell grafts
5601 is 5.0 EU/kilogram body weight, which defines whether a cell graft is pyrogenic or non-pyrogenic [12].
5602 For injectable cell grafts the total amount of endotoxin that can be administered to an adult human of
5603 70 kg should not exceed 350 EU (70 kg × 5 EU). However, the critical amount of endotoxin required to
5604 initiate the sepsis cascade is unclear and depends greatly on the virulence of the infecting organism.
5605 Endotoxin concentrations in clinically septic episodes reportedly range from non-detectable to 12 EU/mL
5606 in plasma, with an approximate mean of 2.5 EU/mL [13].

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Due to the variability in the responses of cells to endotoxins, it is not possible to state the critical level at which endotoxins begin to interfere with the function and growth of cells. The endotoxin limit that can be accepted in cell grafts is based on the route of administration (intravenous or intrathecal), the threshold pyrogenic dose and the volume of the injected cells. For certain cellular grafts, ones that must be administered immediately and that cannot be cryopreserved without damaging the viability and quality of cells, the availability of a rapid testing method for endotoxin testing is fundamental. The bacterial endotoxin test (BET) quantifies only the amount of endotoxin, not the biological impact. Cell-based assays such as the monocyte activation test, an ELISA-based technique which measures release of the pro-inflammatory cytokine 1L-1b (*Ph. Eur.* 2.6.30), might be more suitable for estimating the biological effects of endotoxin [14-16]. The method is highly sensitive (~10 pg/mL of endotoxin) but specificity is compromised as it will also detect non-endotoxin pyrogens. More recently, several electrochemical and optical biosensing techniques using various detection platforms have been developed, some offering high sensitivity (0.01-1 ng/mL) and increased specificity for LPS [17].

10.3.5. Microbiological testing required for specific processing methods

10.3.5.1. Processing using closed systems

For cells in which a closed system is used for processing and for cell therapies where no further cell-cultivation steps are conducted, repeated testing steps are not suitable and do not yield more information on the microbial status of the cell graft. In such cases, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point may be applicable. For microbial testing of hematopoietic progenitor cell preparations, methods need to be validated before use, e.g. matrix validation [18].

10.3.5.2. Processing with terminal sterilisation

For preparations that undergo a validated sterilisation process, the requirements of *Ph. Eur.* 5.1.1 should be considered where methods of sterilisation are described. In particular, it must be shown that adequate precautions have been implemented to minimise the microbial contamination before sterilisation and that tissues and cells with an acceptably low degree of microbial contamination have been used as determined by bioburden testing (see §10.3.6).

Procedures and precautions employed for sterilisation are to be such as to give a sterility assurance level (SAL) of $\leq 10^{-6}$ [19].

If the release of tissues and cells sterilised in their final container is intended to rely on process data only, and not on final tissue or cell testing for sterility ('parametric release'), then validated procedures for all critical production steps and a fully validated sterilisation method must be applied. This approach includes validation of procurement of tissues or cells, transportation, washing, antibiotic treatment and other processing steps, packaging and storage. In some countries authorisation by the competent authority is needed for such an approach.

10.3.5.3. Processing that includes decontamination of tissues and/or cells

Procedures applied for decontamination of tissues and cells are usually limited to approaches in which the vitality and functionality of the preparation can be maintained. For instance, treatment with antibiotics and anti-fungal agents is employed widely to achieve reduction of the microbial load in tissues and cells. Because of virulence mechanisms such as facultative intracellular parasitism, biofilm formation and persistence in a resting metabolic state without growth (which is reversible upon withdrawal of antimicrobial agents or stress conditions), micro-organisms can escape these treatments.

Further processing after the decontamination step should be conducted without antimicrobial agents. Methods for testing of final tissue and cell grafts must be evaluated carefully with respect to possible inhibition of microbial growth due to decontaminating agents or their residues.

10.3.5.4. Open processing without terminal sterilisation

Most tissues and cells, including preparations which have been decontaminated, are exposed to the environment at certain processing stages between procurement and packaging. Without terminal

5655 sterilisation, the environmental contamination risk during open processing must be avoided to the
5656 greatest possible extent. The requirements for microbiological sampling and testing are expected to be
5657 most stringent in these situations, and aseptic conditions must be maintained during procurement,
5658 transportation and the whole manufacturing process.
5659 If open processing takes place without terminal sterilisation, the sampling and microbiological
5660 assessment should include the starting material, the transport solution and any solutions used to wash
5661 tissues and cells. *Ph. Eur.* 2.6.1 provides a means of verifying that the tissues and cells are sterile.
5662 Alternatively, depending on the nature of the tissue or cell-based preparation, the approaches in *Ph. Eur.*
5663 2.6.27 or *Ph. Eur.* 5.1.6 may be applied. If a preparation is not required to be sterile or cannot be rendered
5664 sterile, *Ph. Eur.* 2.6.12 and 2.6.13 can be employed (as described in §10.4.3) allowing quantitative
5665 enumeration of micro-organisms. Such testing may require use of validated methods employing special
5666 media and/or conditions to enable growth of such micro-organisms and their detection. In addition, the
5667 final tissue or cell graft should be tested to ensure quality and safety for clinical use.

5668 **10.3.6. Bioburden testing**

5669 Knowledge is necessary about the acceptable quantitative microbial load of the starting material
5670 before decontamination procedures and/or terminal sterilisation [19]. Bioburden is usually expressed as
5671 a measure of the numbers and variety of micro-organisms on a surface or volume.
5672 For *post mortem* procured human tissues, the quantification of microbial load of the starting
5673 material prior to processing and preservation is an essential prerequisite to inform the potential infection
5674 risk of tissue grafts to an intended recipient. Although bacterial and fungal infection post-transplantation
5675 is rarely reported, some virulent microbes when present in high numbers on a tissue surface may form
5676 cytotoxic exoproducts such as proteases and toxins, which can have a deleterious effect on the cellular
5677 integrity or structural properties of the graft [20-21]. In practice, estimation of bioburden involves:
5678 a. an approximate determination of the numbers of microbes in a tissue or cell sample,
5679 b. identification of the species present, and
5680 c. establishment of acceptance/rejection criteria based on the species identified.

5681 For the assessment of the microbial load of tissues and cells, *Ph. Eur.* test methods 2.6.12 and
5682 2.6.13 can be employed. Where appropriate, tissues or cells can be sampled by a representative tissue
5683 or cell sample suspended in a broth medium and, less commonly, by a culture swab or suspension of an
5684 initial wash of the specimen. Although swab-based methods have a low efficiency of recovery, generally
5685 less than 20% [22-23], they allow a crude estimation of viable microbial contaminants as heavy,
5686 moderate or light and the identification of individual species helps to inform decisions as to the potential
5687 hazard of the contaminant. Rigorous sonication/mechanical shaking methods, exposing representative
5688 tissue samples to an extraction fluid with a surfactant, can increase the recovery efficiency [22].
5689 Coagulase-negative *Staphylococci* and non-pyogenic *Streptococci* are generally the most
5690 frequently isolated organisms [24] and are often susceptible to antibiotic decontamination procedures
5691 [25]. Efficacy studies focusing on the usually expected initial bioburden of tissues or cells, as well as the
5692 type and concentration of effective antimicrobial agents, should be carried out. Based on those studies,
5693 temporary treatment schemes and an exclusion list of specific contaminants for the incoming material
5694 should be determined, based upon not only the category type of tissue but also upon the method by
5695 which the tissue was processed. Table 10.1 provides a list of pathogens that could result in the discard of
5696 the tissues unless they are treated (e.g. cryopreserved musculoskeletal tissues that cannot be "sterilised"
5697 and can only be "disinfected").
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Table 10.1. Pathogenic, highly virulent micro-organisms that could result in tissue discard unless treated with a disinfection or sterilisation process validated to eliminate the infectivity of such organisms [26-29]

Staphylococcus aureus
Pyogenic *Streptococci, Enterococcus* spp.
Non-fermenting micro-organisms: *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomas maltophilia*, *Sphingomonas paucimobilis*, *Burkholderia cepacia*
Sporulating micro-organisms: *Bacillus* spp (*B. anthracis, B. cereus*), *Clostridium* spp.
Enterobacteriaceae (*Escherichia coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp.)
Anaerobic Gram-negative micro-organisms (o.a. *Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp.)
Yeast and filamentous fungi (moulds)
Note: This suggested list is not exhaustive. Some chapters in part B of this guide have a more detailed list of pathogenic, highly virulent micro-organisms for the specific tissues or cells.

10.4. Conditions and methods of microbiological testing

For each procedure, aerobic and anaerobic testing must be conducted under incubation conditions that are appropriate for the detection of tissue- or cell-specific bacteria and fungi (yeasts and moulds) as well as bacteria and fungi of environmental or clinical origin. If indicated, control tests must be carried out for specific micro-organisms that may not be detectable with the culturing media recommended in the relevant chapters of *Ph. Eur.* (e.g. *Mycobacterium spp.*, fastidious micro-organisms).

10.4.1. Sterility testing (*Ph. Eur.* 2.6.1)

Conditions for sterility testing are detailed in Table 10.2. Precautions should be taken against microbial contamination during a test (*Ph. Eur.* 2.6.1). At the least, sub-cultivation should be carried out in a Grade A laminar airflow cabinet, properly disinfected before the test, and no other activity should be conducted at the same time. The preferred microbiological control procedure is dependent on the sample material.

10.4.1.1 Membrane-filtration method

This method uses membrane filters having a nominal pore size ≤ 0.45 µm whose effectiveness to retain micro-organisms has been established.

10.4.1.2 Direct inoculation method

The direct inoculation method is suitable for solutions and tissue samples (i.e. solid substances). Sample quantities are listed in *Ph. Eur.* 2.6.1.

For both methods, microbial growth media are assessed macroscopically for evidence of microbial growth. During incubation, at least one intermediate reading and one final reading should be made. If the test carried out on the sample material results in turbidity of the culture medium, sub-cultivation must be carried out (see *Ph. Eur.* 2.6.1).

Ph. Eur. does not include specific microbiological growth media for the detection of fungi because fungi are detected along with aerobic bacteria in soya-bean casein digest medium. However, other media and /or incubation temperatures may be used, provided that they pass the growth-promotion and validation tests (according to *Ph. Eur.* General Notices on alternative methods). Alternative incubation conditions are shown in Table 10.3.

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5731 **Table 10.2. Incubation conditions for sterility testing**

	Culture medium	Incubation temperature °C	Testing period
Aerobic	Soya-bean casein digest medium (TSB)	20-25	14 days
Anaerobic*	Fluid thioglycolate medium	30-35	14 days
Fungi	Soya-bean casein digest medium (TSB)	20-25	14 days

* Fluid thioglycolate medium will also detect aerobic bacteria.

TSB: tryptic soy broth.

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5733 **10.4.2. Microbiological testing using automated culture systems (*Ph. Eur.* 2.6.27)**

5734 *10.4.2.1. Incubation conditions*

5735 Use of a validated automated culture system may be advantageous, especially for liquid samples
5736 containing cellular material which already results in turbidity of the culture media immediately after
5737 inoculation.

5738 Incubation in automated culture systems should be carried out over at least 7 days. The testing
5739 time can be adapted to specific requirements arising from the characteristics of the preparation. For
5740 example, if risk assessment identifies potentially slow-growing micro-organisms such as *Cutibacterium*
5741 *acnes (Propionibacterium acnes)* the testing time could be extended up to 14-21 days.

5742 The time and temperature of incubation may be too limited to sufficiently account for a broad
5743 range of contaminating micro-organisms found in the environment or in tissues and cells. Hence, the
5744 incubation conditions detailed in Table 10.3 are recommended as alternatives on the basis of risk
5745 assessment, taking into account the expected microbial flora and environmental conditions. Testing
5746 times should be validated.

5747 Preparations of tissues and cells with a short shelf-life may be released based on an intermediate
5748 readout of the test before the test period is completed (negative-to-date result). In the case of a positive
5749 readout during the test period after release of the tissue or cell graft, identification of the microbial
5750 species and a resistogram must be carried out and the information immediately forwarded to the caring
5751 physician.

5752

5753 **Table 10.3. Alternative incubation conditions for microbiology testing**

	Aerobic incubation	Anaerobic incubation
Option 1	20-25 °C normally (automated system), 30-35 °C if necessary (automated system)	30-35 °C (automated system)
Option 2	35-37 °C (automated system); and, where relevant, additional incubation at a lower temperature (manual method) [b]	35-37 °C (automated system)
Option 3	30-32 °C (automated system)	30-32 °C (automated system)
Option 4	30-32 °C (automated system)	35 °C (automated system)

a. Testing period is ≥ 7 days with an automated growth-based method and may be extended up to 14 days. Testing period is 14 days with a manual method.

b. Where relevant, incubate in addition at 20-30 °C. Incubation can be done using commercially available microbiological media, either aerobic bottles intended for automated systems or tryptic soy broth (TSB).

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5755 *10.4.2.2. Sample volume*

5756 For automated culture systems, sample volumes recommended up to 10 mL can be inoculated per
5757 culture bottle. Very small sample amounts of < 1 mL may bear the risk of an increased sampling error,
5758 leading to false-negative results, if only a low microbial count is present in the tissues or cells. Certain
5759 conditions, such as the usual small initial microbial count and early sampling during the production
5760 process or delayed microbial growth in the tissues or cells due to inhibiting substances or unfavourable

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temperature, are reasons for this. Therefore, a large amount of sample should be envisaged for inoculation (if applicable and appropriately validated).

10.4.2.3. Samples without antimicrobial additives

Microbial growth media without any adsorbents, such as resin or activated carbon, should be used. If sensitive organisms are not identified as a possible contaminant in the risk assessment, it is not necessary to add a fastidious organism supplement.

10.4.2.4. Samples with antimicrobial additives

Microbial growth bottles with resin or activated charcoal should be used (if membrane filtration cannot be carried out due to the nature of the sample).

The type, amount and mixture of antimicrobial agents used in manufacturing processes (and therefore present in samples for microbiological testing) is highly variable. Culture bottles containing adsorbing substances are established for the testing of patients' blood with therapeutic doses of a limited number of antibiotics or antimycotics. Therefore, such samples must be validated very thoroughly for residual antimicrobial activity to prove the suitability of the chosen method.

10.4.2.5. Period between inoculation of culture bottles and incubation in an automatic culturing system

Inoculated culture media bottles should be placed into the automated culture system as soon as possible; if a delay occurs, they should be maintained at room temperature [30]. If a delay period of 12 hours is exceeded, the results of the automated culture system must be verified by subculture. In doing so, at least one control smear must be made per negative culture bottle and cultivated under identical conditions. For some automated systems and delayed kinetic culture bottles, manufacturer's specifications mention as acceptable a delay up to 48 hours at room temperature. However, given the variability in terms of delay time, pre-incubation temperature and type of micro-organism, a delay exceeding 12 hours should be validated.

10.4.3. Bioburden testing (*Ph. Eur.* 2.6.12 and 2.6.13)

The tissue-associated bioburden of aerobic mesophilic bacteria and fungi can be quantitatively enumerated either by membrane filtration or plate-count methods according to *Ph. Eur.* 2.6.12. The preferred microbiological recovery procedure is dependent on the type of sample. For example, a representative tissue or cell sample with a known surface or volume can be inoculated in a fixed volume of the sterile extraction fluid 0.1 % peptone supplemented with 0.1 % polysorbate 80. The tissue-containing jar can further be sonicated for 5 minutes (47 kHz) followed by mechanical shaking for 30 minutes (200 strokes/minute) on a linear reciprocal shaker [22].

10.4.3.1. Membrane-filtration method

An appropriate volume of the extraction fluid is filtered through at least two appropriate filters (e.g. 0.45 μM pore size nitrocellulose filter). One filter should be transferred to the surface of a casein soya-bean digest agar plate for determination of the total aerobic microbial count (TAMC) and one filter to the surface of a Sabouraud-dextrose agar plate for determination of the total combined yeasts/moulds count (TYMC). The casein soya-bean digest agar should be incubated at 30-35 °C for 3-5 days and the plate of Sabouraud-dextrose agar at 20-25 °C for 5-7 days. In addition, anaerobic bacteria can be enumerated by transfer of a filter to an appropriate medium plate (e.g. thioglycolate agar) and anaerobic incubation at 30-35 °C for 3-5 days. The microbial count can be calculated as colony-forming units (CFU) per unit weight or volume (gram or millilitre) or surface area of tissues and cells.

10.4.3.2. Plate-count methods

At least two Petri dishes for each level of sample dilution for each medium are prepared, either by the pour-plate method or by the surface-spread method. Plates of casein soya-bean digest agar, Sabouraud-dextrose agar and anaerobic medium agar are incubated as stated above. Plates should be counted which show the highest number of colonies less than 250 for TAMC and 50 for TYMC

5808 corresponding to a given dilution. The arithmetic mean per culture medium of the counts is used to
5809 calculate the number of CFU per unit weight or volume (gram or millilitre) or surface area of tissues
5810 and cells.
5811 For the determination of the absence or limited occurrence of specified micro-organisms, test
5812 conditions are described in *Ph. Eur.* 2.6.13, in which the growth-promoting, inhibitory and indicative
5813 properties of respective media are prescribed.

5814 **10.4.4. Alternative methods for control of microbiological quality (*Ph. Eur.* 5.1.6)**

5815 Alternative rapid microbiological methods (RMM) based on novel technologies that provide
5816 automated, faster and more sensitive microbiological results as compared with classical or conventional
5817 methods may be used to test in-process samples, to demonstrate sterility of cell grafts that have a shelf-
5818 life much shorter than the required testing time for the current compendia sterility test (e.g., 2-5 days
5819 *versus* a 14-day testing period) or in other special circumstances; see Table 10.4.
5820 Such alternative methods must be validated. The validation process includes validation for the
5821 intended use, and equivalence or non-inferiority to the compendia methods.

5822 **Table 10.4. Alternative methods for control of microbiological quality**

Growth-based methods	Electrochemical
	Gas Consumption/Production
	ATP Bioluminescence
	Turbidimetry
Direct methods	Cytometry - Solid phase - Flow cytometry
Cell components methods	Phenotypic
	Genotypic - RT-PCR - Genetic fingerprinting
ATP: Adenosine Triphosphate RT-PCR: Reverse transcription polymerase chain reaction	

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10.5 Validation of microbiological testing methods

5825
10.5.1 Growth-promotion testing

5826 Each batch of the microbiological culture medium used for microbiological testing must be tested
5827 for its growth-promoting capacities as well as being used to test for the microbial strains listed in the
5828 relevant *Ph. Eur.* chapters. In general, it is recommended to include in the assays any possibly relevant
5829 microbial contaminants from the respective tissue preparation or cell preparation or the environment –
5830 for instance, *Cutibacterium acnes (Propionibacterium acnes)* and *Micrococcus* spp. – because of their
5831 specific growth properties.
5832 Growth-promotion testing should also be done for the plates and media used in environmental
5833 monitoring. There should be a formal programme that determines the properties of media for a defined
5834 list of organisms. Growth-promotion testing must show that the media are suitable to consistently

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5835 recover environmental contaminants (if they are present). The standardised list should comprise
5836 organisms based on the literature and/or environmental isolates, and should include a reasonable range
5837 of 'representative' micro-organisms that could be encountered in manufacturing environments (e.g.
5838 Gram-positive rods, Gram-positive cocci, filamentous moulds or yeasts, Gram-negative rods). The list
5839 should contain a minimum of five unique microbial strains [19].

5840 **10.5.2 Method suitability**

5841 The method must be validated in the presence of the intended sample material (e.g. transport
5842 medium, final tissue or cell graft). The basis for method validation is the 'method-suitability test' laid
5843 down in *Ph. Eur., e.g. in chapters Ph. Eur.* 2.6.1 and *Ph. Eur.* 2.6.27.

5844 The same conditions must be chosen as for routine testing (e.g. culture conditions, sample type,
5845 sample amount). The method-suitability test must be undertaken using the bacterial and fungal species
5846 indicated in *Ph. Eur.* 2.6.1. *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* – as
5847 well as the fungi *Candida albicans* and *Aspergillus brasiliensis* – should be incubated under aerobic
5848 conditions; *Clostridium sporogenes* should be incubated under anaerobic conditions.

5849 It is recommended to complement the microbial spectrum by tissue-specific and/or contaminating
5850 micro-organisms such as *Cutibacterium acnes (Propionibacterium acnes)* and *Micrococcus* spp. which
5851 are typical skin contaminants.

5852 For instance, *Cutibacterium acnes (Propionibacterium acnes)* is not readily accessible to skin
5853 disinfection due to its prevalence in the sebaceous glands, and detection of this species in tissue
5854 preparations is not unusual. *Cutibacterium acnes (Propionibacterium acnes)* grows under anaerobic or
5855 microaerophilic conditions as a 'slow-grower' and is associated with particularly long detection time, so
5856 it may be included in method validation. If any other micro-organisms are considered to be relevant
5857 during processing and if present in the environment, they should also be included in validation studies.

5858 The sensitivity of the chosen method should be shown by inoculating 10-100 CFU of the selected
5859 micro-organisms in the presence of the tissues or cells. The microbial count of the dilution used for
5860 inoculation must be verified using a suitable method for each assay (e.g. plating on solid media). If a
5861 method cannot be used to detect microbial counts of 100 CFU, the limit of detection must be evaluated
5862 by experimental studies. Applicability of this method must be assessed in connection with its impact to
5863 ensure microbial safety of the tissues and cells.

5864 Each micro-organism species should be tested. For evaluation of the robustness of the method, it
5865 is recommended that testing of the same organisms is repeated at different time points (independent
5866 experiments) and that assays are repeated in the same way with defined deliberate variations (different
5867 staff, batches of consumables, and days).

5868 For comparison, a positive control (without tissues or cells) must be included in the test for each
5869 test strain. For negative control, a suitable amount of sample of the tissues or cells to be tested must be
5870 incubated in the aerobic and anaerobic culture medium, at least in duplicate without inoculating micro-
5871 organisms. Positive and negative controls must also be prepared, ready for use in the event of repeat
5872 tests.

5873 Inoculated media must be incubated under the conditions applied in routine testing (temperature,
5874 duration) and checked for growth at regular intervals.

5875 Test assays and controls must be evaluated in predetermined intervals during and at the end of the
5876 testing period. Samples for subculture must be taken from positive detected tests as quickly as possible.
5877 In the case of microbial growth, the micro-organisms must be identified.

5878 If inhibition of microbial growth by the sample material is identified at validation, the method
5879 must be adapted in an appropriate way, for instance, using a higher volume of the culturing media or
5880 addition of binding or enzymatic substances that inhibit antimicrobials.

5881 **10.5.3 Documentation and interpretation of results**

5882 All materials used and working steps undertaken must be documented. Interpretation of results 5883 should include at least the following factors:

5884 a. assessment of the growth of micro-organisms in the presence of the tissues or cells to be tested 5885 and in controls;
5886 b. specification of the microbial count for evaluation of the method;
5887 c. period of time until a positive result has been detected for test assays and positive controls;
5888 d. proof of identity of inoculated micro-organisms.

5889 For negative controls or test assays without detection of micro-organisms, the total testing period 5890 and results of the subculture (including the methods used) must be specified.

5891 10.6 Interpretation of results and actions to be taken

5892 In general, source material that demonstrates contamination must be rejected unless the preparation 5893 undergoes decontamination and/or terminal sterilisation, and the detected quantity and quality of micro-5894 organisms can be reliably inactivated or removed by the intended procedure, or if it is justified by 5895 exceptional clinical circumstances (refer to Part B for specific examples).

5896 Contaminated source material should be rejected if processing includes decontamination (but not 5897 terminal sterilisation) and if risk assessment considering the intended route of administration cannot 5898 exclude risk to the recipient even if adequate antimicrobial treatment is initiated. Such source materials 5899 should be evaluated on the basis of qualitative (exclusion list for objectionable micro-organisms) and 5900 quantitative (microbial count, bioburden) microbiological control tests, and specifications should be 5901 given. The decontamination procedure should be shown to be suitable to remove or destroy the type and 5902 number of contaminants allowed in the source material. In particular, multi-drug resistant micro-5903 organisms – e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant 5904 *enterococci* (VRE) and extended-spectrum beta-lactamases (ESBL) – and possible toxin-producing 5905 micro-organisms, such as *Pseudomonas aeruginosa*, *Streptococcus* group A, *Staphylococcus aureus*, 5906 *Clostridium* and *Bacillus,* as well as yeasts and filamentous fungi, need to be evaluated carefully, and if 5907 appropriate, the tissues and cells should be rejected.

5908 In the case of locally acquired contamination or a local infection, the microbiological result 5909 applies only to the tissue where the contamination was detected and to tissues that could have been 5910 cross-contaminated. If bacteraemia, septicaemia (anamnestic or blood culture) or any other distribution 5911 of the objectionable micro-organisms (at procurement, storage, transport, manufacturing) cannot be 5912 excluded, other tissues should be rejected.

5913 For contaminated autologous preparations, or preparations received from a specific allogeneic 5914 donor, whereby a repeated procurement cannot be conducted or involves a high degree of risk, risk 5915 assessment based on the urgency of the application, judgment of infection risk and treatment options 5916 must be conducted. In any application of such preparations, measures must include full identification of 5917 the contaminating micro-organism and their resistograms, as well as adequate prophylaxis of the 5918 donor/recipient if the tissues or cells must be used.

5919 10.7 General considerations for environmental microbiological 5920 monitoring

5921 Guidance for planning and carrying out environmental monitoring of cleanrooms, clean zones, 5922 processing methods and other equipment is described in Chapter 7.

5923 Microbial samples can be taken using four sampling methods: volumetric air sampling, settle 5924 plates, contact plates and glove prints – or fingerprints. A non-selective culture medium, permitting 5925 growth of the expected micro-organisms, and containing additives to overcome the residual effect of

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5926 biocides and cleaning agents, should be selected. Additives inhibiting residual biocides and cleaning
5927 agents are an essential component of the culture medium.
5928 At present no commercial neutraliser is able to inactivate all biocides. The choice for a sanitation
5929 and disinfection programme with a specific cleaning agent and biocide must be well considered. The
5930 concentration of residue left on the surfaces after cleaning depends on the type of biocide and the
5931 sanitation programme. The culture medium used for environmental monitoring has to be appropriately
5932 validated for the growth of diverse bacteria and fungi, and it must be possible to demonstrate that the
5933 residues generated by the sanitation programme do not interfere with micro-organism recovery. Table
5934 10.5 summarises the characteristics of these sampling methods.
5935

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5937 **Table 10.5. Environmental microbiological monitoring methods**

Method	Air or surface, qualitative or quantitative	Notes
Volumetric sampling	Air quantitative	Can be used to quantify bacteria and fungi suspended in the air in direct contact with the tissues or cells. Active sampling can be used to detect homogeneous suspensions of micro-organisms in the air, but it is not a reliable measure of the sporadic contamination that occurs during operations. The chosen device should be shown to correspond to current standards of sensitivity and detection. Sample sizes should be taken for each measurement in function of the bacterial load of the environment. Preferable sample sizes of 1 m³ should be taken for Grade A and B areas. If this sample size results in an unreadable number of CFU (e.g. Grade C and D areas), reduced volumes may be employed if justified. The effect of capture-plate drying during sampling and transport to the microbiological laboratory should be determined by a validation study. Time limits should be set to ensure that micro-organisms remain viable up to the point of transfer to an environment for detection of growth promotion.
Settle plates	Air qualitative	Can be used to detect bacteria and fungi that descend in a column of air over the plate during the process. Need to determine how long the plates can be open (usually 2-4 hours). Exposed plates may be replaced by unused ones so that total time of exposure is reached.
Contact plates	Surface qualitative	Contact plates can be used to detect contamination by micro-organisms in the immediate vicinity of an area (e.g. working area, furniture, clothes, equipment, walls, ceiling, materials). The pressure and duration of contact have a significant influence on microbial yield (recommendation: uniform pressure for 10 seconds). Tested surfaces must be cleaned after sampling.
Swabs	Surface qualitative	Used for wiping of surfaces that cannot be sampled with contact plates. A swab dampened with sterile physiological (0.9 %) NaCl solution is used to test dry surfaces. A dry swab is used to test damp surfaces. The sample material collected is then wiped onto an agar plate. The sample area should be ≈ 25 cm^2, if possible. Tested surfaces must be cleaned after sampling.
Glove prints	Glove or fingertips qualitative	Fingertips are the most likely area to come into contact with microbial contamination on work surfaces, on materials or arising from the operator and then transferred onto tissues or cells. Glove prints (all five fingers) of both hands should be taken to assess this possibility. Usually placed on contact plates after aseptic processing or before changing gloves. Gloves must not be disinfected before samples are taken. A firm and even pressure should be applied for ≈ 5-10 seconds, taking care not to damage the agar surface.

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5939 ### 10.7.1 Incubation of samples

5940 Environmental monitoring samples should be incubated at a minimum of two different
5941 temperatures to detect bacteria and fungi. Incubation conditions for environmental microbiological
5942 testing are detailed in Table 10.6. Incubation for 3-5 days at 20-25 °C followed by incubation at 30-35 °C
5943 for an additional 2-3 days has been shown to be sufficient to detect most bacteria and fungi. The method
5944 chosen should be validated and standardised very carefully. Alternative methods are acceptable if high
5945 recoveries (> 90 %) of micro-organisms of interest can be demonstrated consistently [31].
5946 If micro-organisms are expected in the environment, and cannot be detected using standard media
5947 for environmental monitoring with the temperatures recommended above, the procedure must be
5948 adapted accordingly.
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5951 **Table 10.6. Incubation conditions for environmental microbiological monitoring**

	Culture medium	Incubation temperature	Testing period
Aerobic Fungi	Trypticase soy agar irradiated	20-25 °C + 30-35 °C	3-5 days + 2-3 days
Alternative incubation conditions			
Aerobic*	Trypticase soy agar irradiated	30-35 °C	2-3 days
Fungi	Sabouraud agar	20-25 °C	5-7 days

* When applicable, consider also anaerobic testing in the same culture conditions as aerobic testing but in anaerobic atmosphere.

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5953 ### 10.7.2 Data analyses

5954 Reading of plates should be done according to a defined, standardised procedure. Identification
5955 of CFU should be undertaken according to the environmental monitoring programme of the tissue
5956 establishment. According to EU GMP, detected CFU in Grade A areas must be identified to the genus
5957 or species; in Grade B areas, detected CFU should be identified.

5958 ## 10.8 References

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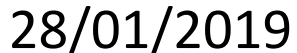

Chapter 11. Distribution and import/export

11.1. Introduction

This chapter describes the requirements for distribution of tissues and cells including unfinished products and defines recommended controls for their import and export. The term 'distribution' should be understood to mean transport and delivery of tissues or cells intended for human application. The term 'unfinished' should be understood to describe tissues and cells just after procurement or during processing. 'Transport' is meant as the act of transferring a tissue or cellular product between facilities under the control of suitably trained, designated and authorised staff at the distributing and receiving facilities. 'Shipment' is a type of transport where the transfer of the tissues or cells from the distributing to the receiving facility is carried out by means of a contract with a third party, usually a specialised logistics company. The entire distribution chain must be validated appropriately, including the equipment used, to ensure the maintenance of critical transport or shipment conditions.

The terms 'import' and 'export' should be understood to include all processes and procedures that facilitate the entry or exit of tissues and cells, whatever their step of processing, to/from a single country. Import/export controls must ensure that the quality and safety of the tissues or cells are in compliance with this Guide.

Tissues and cells can be transferred by a tissue establishment:

- to a clinical facility within the same country, where they will be applied (i.e. distribution);
- to another tissue establishment within the same country for local distribution.

Cross-border movement of tissues and cells includes transfers:

- to a tissue establishment outside the country (i.e. export);
- from another country to a clinical facility or tissue establishment in the country (i.e. import).

For transfers of tissues or cells between countries that are within the European Union (EU), usually referred as 'distribution', the legislation does not require import/export controls to be in place provided that these tissue and cell preparations come from a duly authorised tissue establishment and their processes have also been authorised by the national competent authority. However, several EU member states opt to apply more stringent requirements than those in the directives and consider this movement in the same way as import/export involving countries outside the EU (referred to as 'third countries'). Written agreements might be needed between countries in some member states (see §11.5.5).

11.2. Release

Prior to distribution, a comprehensive record review should ensure that all elements of collection, processing and storage have met the established quality criteria, including identity of the product. In a case of incomplete eligibility of the donor, the product must be released only for documented urgent medical need (see §12.5 on release). An alternative plan of transport or shipping should be available in case of emergency situations, to prevent possible clinical complications to the recipient. The courier should be able to contact the receiving facility on a 24-hour basis in case of delay during transit.

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11.3. Transport

The choice of mode of transport should take into account any general regulations governing transportation of biological substances.

Critical transport conditions, such as temperature and time limit, must be defined to ensure maintenance of the required properties of tissues or cells [1]. When transport is carried out under storage conditions, the impact on transport time is minimal. Unfrozen products are usually transported refrigerated (2 to 8 °C), or cooled (8 to 15 °C) or at room temperature (15 to 25 °C); frozen products are transported deep-frozen (−80 to −60 °C in dry ice), frozen (<−15 °C with ice packs), cryopreserved (<−140 °C in liquid nitrogen vapour phase). When the transport device does not allow the temperature conditions to be maintained over time, a time limit must be validated to guarantee that the storage conditions during transport do not affect the quality of the product.

For unfrozen products, such as bone marrow, there are conflicting recommendations for storage and transportation – e.g. 4 °C *versus* room temperature [2, 3, 4] – so the transplant centre is normally requested to define the transport conditions they wish to be applied. 'Room temperature' should be defined as a controlled temperature range with defined values for the upper and lower limits. Special attention should be paid to shipment at room temperature when the planned journey length is over 6 hours. Special attention should also be paid to refrigerated conditions: when the pharmacopoeia sets the temperature range between a lower limit at +2 °C and an upper limit at +8 °C, the risks of damage should be considered for cells or cornea exposed at +2° C temperature [5, 6, 7]. For cells and tissues potentially contaminated during the procurement, refrigerated transportation is generally recommended in order to prevent the risk of bacterial proliferation.

If the tissues or cells require specific environmental conditions, the capacity of the transport container to maintain the required environmental conditions, and the length of time that these conditions can be maintained by the transport container, should be determined by validation and documented. For instance, if liquid nitrogen is used to maintain very low temperatures, the dry-shipper shall contain sufficient absorbed liquid nitrogen to maintain the storage chamber temperature <− 140 °C for a defined period of time, at least 48 h beyond the expected time of arrival at the receiving facility. Where temperature control is critical, data loggers should be used to monitor temperature during transport or shipment, with data downloaded from the device providing a graph to show that temperature was within the acceptable range at all times. Temperature indicators can be also used to indicate exposure to extremes of temperature.

Containers/packages should be secured and labelled appropriately (see Chapter 14).

Written agreements should be in place for the shipment of tissues and cells. In EU member states a written agreement must be signed between the shipping company and the tissue establishment to ensure that the required conditions will be maintained. This document must describe what should happen if the tissues or cells are damaged or lost during shipment (see also Chapter 2) and must cover a requirement that any related serious adverse events should be identified and reported to the Health Authorities (see Chapter 16).

11.4. Allocation

The allocation of tissues and cells should be guided by clinical criteria and ethical norms. The allocation rules should be equitable, externally validated and transparent.

The procedures for distribution of tissues and cells by authorised tissue establishments must comply with the criteria laid out in the sections below.

It is mandatory for EU member states to have procedures in place for the management of requests for tissues and cells. The rules for allocation of tissues and cells to certain patients or healthcare institutions must be documented, and made available in appropriate circumstances, in the interests of transparency.

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6108 **11.4.1. Visual examination**
6109 Packaged tissues or cells should be examined visually for appropriate labels, expiry date,
6110 container integrity and security, and any evidence of contamination prior to being dispatched (see
6111 Chapter 14).

6112 **11.4.2. Medical competence**
6113 Distribution for clinical application should be restricted to hospitals, physicians, dentists or other
6114 qualified medical professionals and must comply with all applicable national regulations.

6115 **11.4.3. Documentation**
6116 The place, date and time of pick-up and delivery (including time zone where relevant) and identity
6117 of the person receiving the tissues and cells should all be recorded, and this record should be maintained
6118 in the tissue establishment from which the tissues or cells are distributed.
6119 Any transportation must be accompanied by specific documentation attached to the package (see
6120 Chapters 6, 12 and 14).

6121 **11.4.4. Recall and return procedures**
6122 An effective recall procedure must be in place in every tissue establishment, including a
6123 description of the responsibilities and actions to be taken in the case of a recall. This must include
6124 procedures for the notification of the relevant Health Authority/ies and all the facilities/institutions
6125 potentially affected by the recall.
6126 A documented system must be in place for the handling of returned products, including criteria
6127 for their acceptance into the inventory, if applicable.
6128 For further information see Chapter 15.

6129 ## 11.5. Import and export

6130 **11.5.1. Underlying principles**
6131 Import and export between countries should be done only through legally authorised tissue
6132 establishments that can guarantee that they have sufficient competence to evaluate safety and quality
6133 and also can guarantee that they have adequate systems to meet traceability requirements. They should
6134 be specifically authorised for one or more of the following:
6135 a. import and/or export of human tissues and/or cells intended for human application;
6136 b. import and/or export of tissues or cells intended for the manufacture of medicinal products derived
6137 from human tissues and/or cells (with the exception of tissues/cells that have been substantially
6138 manipulated, such as cell-lines or cell banks);
6139 c. import of procured human material intended for processing, storage or banking in a tissue
6140 establishment or cell establishment in their country.

6141 As a general rule, if organisations responsible for human application, manufacturers of advanced
6142 therapy medicinal products, clinical practitioners or individuals identify a need to import tissues or cells,
6143 they should organise this through a written agreement with a licensed tissue establishment in their own
6144 country. Third-party agreements must specify the terms of the relationship and the relevant
6145 responsibilities, as well as the protocols to be followed, to meet the required performance specifications.

6146 **11.5.2. Import**
6147 Tissue establishments that wish to import tissues or cells should be able to demonstrate that the
6148 need cannot be adequately met by comparable material available from sources within their country or
6149 that there is another justifiable reason for the import. They should also be able to justify the import in

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6150 terms of accessibility, quality, speed of supply, risk of infection, quality of service, cost-effectiveness or
6151 scientific or research needs. They should ensure that any material intended for import is consistently
6152 sourced under the legal and ethical requirements of their country and the exporting country. If the
6153 importing tissue establishment cannot satisfy itself that ethical standards are in place in the country of
6154 origin, the tissues or cells should not be imported.
6155 The safety and quality characteristics of the tissues or cells to be imported should be equivalent
6156 to those in place within the importing country. Imports should be accepted only from countries that have
6157 established procedures to authenticate the legitimacy of exporters and the provenance of the donated
6158 material they supply. Exporters should be asked to provide evidence of compliance with the regulations
6159 that they are required to observe before any orders are placed with them.
6160 Companies that act as distributors, often also carrying out import and export activities, have
6161 responsibilities equivalent to those of tissue establishments for ensuring the equivalent safety and quality
6162 requirements, for maintaining traceability and for having adequate vigilance systems in place.
6163 Fulfilment of these requirements implies having suitably trained, designated and authorised staff
6164 (including those with medical expertise) to evaluate donor-selection criteria and reports of adverse
6165 incidents and reactions.
6166 See also § 11.5.5 below for EU requirements for import.

6167 *11.5.2.1. Routine importation*

6168 The importing tissue establishment should assess whether the supplying tissue establishment
6169 complies with the quality and safety recommendations in this Guide and document that assessment,
6170 which includes respect for the fundamental ethical principles of consent, non-remunerated donation,
6171 anonymity and respect for public health. The evaluation should include at least the following:
6172 a. the general quality and safety systems at the exporting establishment, including organisational
6173 chart, staff training, facilities, processing methods, validation studies, traceability and biovigilance
6174 systems, licences and accreditation (including lab certification/authorisation) and donor blood
6175 testing;
6176 b. a review of the safety and quality of individual dispatches of tissues or cells (i.e. confirmation of
6177 donor consent, verification of donor sample testing and the results, donor eligibility records,
6178 description of the tissue or cells, transportation arrangements, etc.).

6179 Potential language barriers should be considered and a common language agreed upon for all
6180 donor and tissue- and cell-related documentation.
6181 A service-level agreement or contract between the exporting and importing tissue establishments
6182 that clearly defines roles and responsibilities is a basic requirement. Agreed procedures for the transport
6183 of the tissues and cells from the country of origin to the tissue establishment in the importing country
6184 should form part of the contract and should specify the methods to be followed to ensure maintenance
6185 of the required environmental conditions, of the package integrity and of compliance with agreed
6186 timeframes. Such transportation should be direct, without intermediate stops when possible, using an
6187 approved courier. The courier or transportation service must provide records of pick-up and delivery to
6188 the tissue establishment so that complete traceability is ensured.
6189 The agreement should specify how tissues and cells will be identified. Unique identifying codes
6190 should allow traceability and a formal and unambiguous identification of all tissues and cells (see
6191 Chapter 14).
6192 Agreements between importing tissue establishments and suppliers in other countries should
6193 include provisions for the performance of audits at the exporting facility and should require that any
6194 changes to authorisation status be immediately communicated to the importing tissue establishment.

6195 *11.5.2.2. 'One-off' importation*

6196 There may be cases where exceptional or one-off importation is necessary for a single patient. In
6197 these cases, the importing tissue establishment should ensure that there exists a documented evaluation

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6198 of the safety and quality of the tissues or cells being imported. The importing tissue establishment should
6199 keep the documentation obtained from the supplying tissue establishment for the time period specified
6200 in national regulations (e.g. 30 years in EU member states).
6201 In limited cases (e.g. in emergency situations or for immediate transplantation) the import of
6202 certain tissues and cells may be directly authorised by a Health Authority, which should take all the
6203 necessary measures to ensure that imported tissues and cells respect the national quality and safety
6204 standards.

6205 **11.5.3. Customs and security clearance**

6206 For clearance of Customs, all tissues and cells supplied from abroad require a clear description of
6207 the content of the consignment and its destination and must be labelled as described in Chapter 13. It is
6208 important that frozen tissues or cells, which are usually packed in dry ice or stored in a dry-shipper, as
6209 well as fresh cells and tissues for urgent medical need, must not be delayed at border crossings. Viable
6210 tissues and cells for clinical use must not be exposed to irradiation devices; instead they should be subject
6211 to a visual inspection.
6212 However, it should be noted that a study published in 2002 concluded that even 10 passages
6213 through the hand-luggage control system resulted in no harm to haematopoietic progenitor cells (HPC)
6214 and lymphocytes in terms of viability and potency. Interestingly, the radiation dosage of passage through
6215 the hand-luggage control system is of 1.5 + 0.6 μSv compared to a radiation dose of 60 μSv received by
6216 the HPC during a 10 h flight [8]. The lack of data on long-term effects suggests that, in line with the
6217 precautionary principle, the non-irradiation rule should be followed for the time being. Therefore, it may
6218 be expedient for the importer to inform Customs of a prospective consignment and any enquiries by
6219 Customs should be answered promptly. The agreement with the exporter should define responsibilities
6220 for meeting the cost of transport, refrigeration and/or storage at a Customs facility for any items that
6221 may be detained pending Customs enquiries.

6222 **11.5.4. Acceptance at the tissue establishment**

6223 Each importing establishment should have a documented procedure and specifications against
6224 which each consignment of tissues and cells, together with its associated documentation, is verified for
6225 compliance with the written agreement in place with the exporter. Any non-compliance should be
6226 reported to the exporter. Consignments should be examined for any evidence of tampering or damage
6227 during transport.
6228 Tissues and cells should be stored in quarantine in an appropriate secure location under defined
6229 conditions until they, along with their associated documentation, have been verified as conforming to
6230 requirements. The acceptance or rejection of received tissues and cells should be undertaken and
6231 documented in accordance with the guidance shown in Chapter 12.

6232 **11.5.5. EU requirements for importing tissues and cells**

6233 In April 2015, a new implementing directive on procedures for verifying equivalent standards of
6234 quality and safety of imported tissues and cells was adopted by the EU. Commission Directive
6235 2015/566/EU stipulates that tissues and cells must be imported into the EU by an importing tissue
6236 establishment authorised for such imports by competent authorities. An importing tissue establishment
6237 is defined in the directive as:

6238 *"a tissue bank or a unit of a hospital or another body established within the Union which is a party to a*
6239 *contractual agreement with a third country supplier for the import into the Union of tissues and cells coming*
6240 *from a third country intended for human application."*

6241 Directive 2015/566/EU also lays down the obligations of the importing tissue establishments and
6242 the competent authorities of EU member states who need to verify that imported tissues and cells meet
6243 quality and safety standards equivalent to those in place in the EU legislation for tissues and cells. These

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new requirements aim to facilitate the exchange of tissues and cells with non-EU countries while ensuring high standards of quality and safety are applied whatever the origin of the imports.

The procedures laid down in the new directive mirror closely the verification systems already in place within the EU. That is, procedures on the authorisation and inspection of importing tissue establishments are laid down, specifying the information and documentation that needs to be provided or made available to Health Authorities in EU member states when tissue establishments apply for import authorisations. Such information and documentation relates to the importing tissue establishment itself and the non-EU country suppliers it plans to use as a source of tissues and cells.

Another key element of the 2015 directive concerns the need for written agreements between importing tissue establishments and their non-EU-country suppliers. Several minimum requirements for such agreements are listed in the text with a view to ensuring that the roles and responsibilities of each party are clear and fully undertaken to ensure equivalent quality and safety standards are met. Annexes to the directive describe the minimum requirements in the information and documentation to be provided by importing tissue establishment applicants when applying to be accredited, designated, authorised or licensed for the purpose of import activities, the content of the authorisation certificate for importing tissue establishment and the information to be provided regarding the third-country supplier.

The directive allows a limited number of exceptions to certain procedures for situations where certain tissues and cells are imported on a one-off basis. A ‘one-off import’ is defined in the directive as

> *“the import of any specific type of tissue or cell which is for the personal use of an intended recipient or recipients known to the importing tissue establishment and third country supplier before the importation occurs. Such an import of any specific type of tissue or cell shall normally not occur more than once for any given recipient. Imports from the same third country supplier taking place on a regular or repeated basis shall not be considered to be ‘one-off imports”*.

Those tissues and cells imported under direct authorisation of the competent authority of an EU member state (i.e. in emergency situations or for immediate transplantation) are not affected by the new procedures. An ‘emergency’ is defined in the directive as

> *“any unforeseen situation in which there is no practical alternative other than to urgently import tissues or cells from a third country into the Union for immediate application to a known recipient or known recipients whose health would be seriously endangered without such an import.”*

In the EU, distribution and shipment of all cells classified as advanced therapy medicinal products (ATMP) are within the responsibility of a marketing authorisation holder and supervised by national/EU authorities for medicinal products.

Where an EU country imports from a non-EU country and the ultimate destination is a different EU member state, then the tissues or cells should fulfil the quality and safety requirements of both EU countries (i.e. with one EU country acting as the point of entry into the EU and the other as the final receiver of the tissues or cells).

11.5.6. Export

Tissues or cells should not be exported if there is an unmet clinical need for the material in the country of origin. Exported material should be procured, used, handled, stored, transported and disposed of in accordance with the consent that has been given by the donor. Tissues and cells should be exported only to countries that have proper controls on the use of donated material. They should be exported only for the purposes for which they can lawfully be used in the country of destination, and exporters should satisfy themselves beforehand that the human tissues and/or cells will be used for a *bona fide* clinical application or research.

Tissue establishments should ensure that the quality and characteristics of the tissues and cells to be exported are equivalent to those of the tissues and cells implanted in their own country and are required in the country of destination.

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6291 ## 11.6. International co-operation

6292 For some transplant patients, including sensitised patients, it may be difficult to find a match within their
6293 own country. In these cases, co-operation between countries is necessary and in some cases it may be
6294 necessary to search worldwide to identify suitable donors. International co-operation and exchange of
6295 tissues and cells is necessary to increase the chances of providing tissues and cells for patients in life-
6296 threatening situations. For these reasons, it is important to ensure that there is good co-operation between
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Chapter 12. Organisations responsible for human application

12.1. Introduction

An organisation responsible for human application (ORHA) is a healthcare establishment or a unit of a hospital or another body that carries out human application of tissues or cells. Once tissues and cells, which must be ordered by a clinician or other authorised person, arrive at an ORHA, the responsibility for maintaining the quality-assurance chain is transferred to that organisation. The ORHA must store and handle tissues and cells correctly according to the instructions of the supplying tissue establishment (TE). The ORHA must also maintain traceability and biovigilance, which includes responsibility for immediately reporting serious adverse reactions and events to the TE and to the Health Authority, participating in the investigation and, where required, implementing corrective and preventive actions. This chapter is based on the guidance Vigilance and Surveillance of Substances of Human Origin (SoHO V&S) published by the EU and on the basis of good practice that has been described in the American handbook for practitioners [1, 2, 3].

12.2. Decision on using and ordering tissues and cells

A serious adverse reaction (SAR) is defined in EU Directive 2004/23/EC as an unintended response that is fatal, life-threatening, disabling or incapacitating, or which results in, or prolongs, hospitalisation or morbidity. The risk of an SAR in a recipient of tissues and cells is considered to be very low, especially if the tissues and cells have been highly processed or terminally sterilised. However, the human application of tissues or cells is not free of risk and examples of SARs are documented in the World Health Organization's Notify Library [4], hosted by the Italian National Transplant Centre. Clinicians must, therefore, give careful consideration to the risks and benefits of the human application of tissues and cells, and the feasibility and availability of alternative options.

Somatic tissues and cells for allogenic application are donated altruistically for the benefit of patients in need and are often in short supply; therefore, only the required amount should be ordered, to minimise the likelihood of wastage. Healthcare professionals responsible for the storage and preparation of human tissues and cells for clinical application should receive appropriate training to ensure their compliance with all applicable technical and legal requirements that assure the quality and safety of the supplied tissues and cells.

12.3. Choosing a supplier of tissues or cells

In most cases, procured tissues and cells require processing and storage at the TE before their distribution to ORHAs for human application. In cases of direct distribution, procurement organisations send procured tissues and cells directly to the ORHA for immediate transplantation without any intermediate steps such as processing or storage.

Before requesting tissues or cells, the ORHA should confirm that the supplying TE, or the procurement organisation in the case of direct distribution, is compliant with all relevant legal and technical standards and requirements for the lawful provision of tissues and cells that are safe and of appropriate quality. In the case of procurement of haematopoietic progenitor cells (HPC) or lymphocytes for unrelated allogeneic use, there is no direct interaction between the procurement organisation and the ORHA before cell procurement. This means that the ORHA does not directly select the procurement organisation that will procure the HPC or lymphocytes. Suitable donors are identified through donor

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registries. The registry is responsible for ascertaining whether the procurement organisation complies with appropriate quality and safety standards, including traceability of the procured cells.

Tissue establishments must be authorised, accredited, designated or licensed (collectively referred to in this chapter as 'authorised') by an appropriate Health Authority. This authorisation must specify the types of tissues or cells that can be accepted and the permitted activities, including procurement, donor testing, processing, storage and distribution, undertaken by the TE. The TE must be inspected regularly by the Health Authority to confirm compliance with legal requirements and quality and safety standards. Health Authorities must also authorise, where appropriate, the direct distribution of tissues and cells to ORHAs for immediate clinical application from abroad.

Using only appropriately authorised TEs ensures that the donors of tissues or cells have been selected and tested correctly, and that all quality system requirements are in place for the procurement, processing, storage and distribution of tissues or cells. ORHA may consider it appropriate to conduct a quality audit of a supplying TE.

To ensure that the quality and safety standards and the respective responsibilities of TEs and ORHAs are clearly set out and fully understood by both parties, there should be a formal service-level agreement (SLA) or contract in place between the supplying TE and the ORHA. These written agreements should be signed, dated and reviewed at least annually, but sooner if changes are required. They must comply with relevant laws and regulations. Where an ORHA and the supplying TE are within the same healthcare institution, responsibilities should be specified in the overall quality-system documentation.

Service-level agreements should include:

a. contact details for relevant persons in both parties, including the TE's Responsible Person (RP);
b. procedures for ordering and the delivery of tissues or cells, including liability for transport;
c. a statement that storage and preparation of tissues and cells for human application at the ORHA must comply with all relevant and specific instructions provided by the TE, including adherence to expiry dates;
d. procedures at the ORHA for the lawful disposal of unused tissues or cells or remnants of tissues and cells after human application;
e. procedures, if permitted, for the return of tissues or cells to the TE;
f. responsibility for maintaining traceability and biovigilance, including procedures for the timely reporting and investigation of adverse reactions and adverse events, including 'near misses', and procedures for the management of tissue and cell recalls and look-backs;
g. procedures, where permitted, for reporting of relevant clinical outcome data relating to the quality, safety and efficacy of the applied tissues or cells by the ORHA to the TE (see §12.14).

Where novel tissues and cells are to be supplied by a TE, or where novel clinical applications of consolidated tissues and cells are intended, these should be authorised by the Health Authority. The extent of clinical follow-up needed to evaluate the effectiveness of the applied tissues and cells should also be agreed between the Health Authority, TE and ORHA (see Chapter 29).

It is strongly recommended that ORHAs should obtain tissues or cells directly through a TE. If a broker (meaning here: an organisation that mediates for a payment between an ORHA and a TE in tissue and cell distribution) is used, the ORHA must verify that the distributing TE is authorised appropriately, that the broker has an agreement signed with the TE, and that the tissues and cells are supplied on a non-profit basis from voluntary unpaid donations.

Tissue establishments should only distribute gametes, embryos and germinal tissue to other authorised TEs or ORHAs for human application under the supervision of a clinician or other appropriate healthcare personnel. Distribution to individuals should be avoided.

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12.4. Importing tissues or cells from other countries

If an ORHA needs to import tissues or cells from another country, it is good practice to make arrangements through a local TE to locate and communicate with the exporting TE. In the EU, tissues and cells from a third country outside the EU must be imported through a TE authorised for importation by an EU Health Authority. Commission Directive 566/2015/EU sets out the procedures for verifying the equivalent standards of quality and safety of tissues and cells imported from third countries. The only exceptions to this rule are tissues and cells imported for direct distribution or for cases where there is an urgent clinical need. In the former, imported tissues and cells may be distributed directly for immediate clinical application provided the supplier is authorised for this activity. Urgent clinical cases include any unforeseen situation where there is no alternative other than to urgently import tissues and cells from a third country for immediate application to a known recipient or known recipients whose health would be seriously endangered without such an import. In both of these cases, the Health Authority must authorise the import directly.

The importing and exporting TEs must liaise with each other to ensure that the equivalent standards of quality and safety required by the importing TE are applied. In the EU, any TE that is authorised by a Health Authority in its own member state may provide tissues or cells directly to ORHAs in other member states. However, some member states have implemented more stringent regulations that require formal import procedures to be followed, even if the tissues and cells come from another EU member state. It is important to be aware of the national legislation in place for the importation of tissues or cells from another country.

12.5. Exceptional release

In exceptional circumstances, an ORHA may agree with a TE that tissues or cells that do not meet the normal release criteria can be applied in a specific individual on the basis of a risk–benefit analysis, taking into consideration the alternative options for the individual and the consequences of not providing the tissues or cells. The risk assessment should be documented before acceptance of the exceptionally released tissues or cells. The recipient's clinician should liaise with the TE's RP in conducting the risk assessment and risk–benefit analysis for the intended recipient. These discussions and conclusions should be documented. The treating clinician should sign an agreement accepting clinical responsibility for the exceptional release. The recipient, where possible, must be informed as part of the normal consenting procedure of the intention to use tissues or cells under exceptional release (see §12.6).

12.6. Recipient consent

Although donors are carefully selected and tested, there remains an albeit small risk of an adverse reaction in a recipient of tissues or cells. Recipients must therefore be made aware both of the risks and benefits of the intended treatment to be able to give informed consent. The Notify Library is an invaluable source of information for clinicians when evaluating the risks associated with the human application of tissues and cells [3]. Where the collection of clinical follow-up data is proposed, recipients may need to provide consent for the sharing and secondary use of their data in accordance with national legislation and guidance. In the EU, the General Data Protection Regulations (GDPR, Regulation EU 2016/679) provide certain exemptions from the need for consent for the collection and use of such data under Articles 6.1(e) and 9.2(h). However, recipients must be informed about the collection and use of their clinical data and, although they do not need to give consent, they do have the right to refuse to allow their data to be collected and used. Moreover, they can request to have their data withdrawn at any time even though they may not initially have refused their permission.

The information given to a prospective recipient should include at least the following:

a. a description of any adverse outcomes that have been reported for the given type of tissue or cell application;

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b. an estimate of the frequency of the adverse outcomes described;
c. whether the treatment is consolidated or if it involves novel methods of processing/clinical application;
d. information on alternative treatments, if available.

Once the appropriate information has been given, the recipient, if willing to proceed, should then consent to the treatment, according to national requirements. The recipient should confirm:

a. that the risks associated with the human application of the tissues or cells have been explained and the information has been understood;
b. that they accept the risks in light of the potential benefits.

It is strongly recommended that a specific consent form should be signed by the recipient in the case of any novelty, both at the TE, such as introduction of new preparation methodology, as well as at the ORHA, such as introduction of new clinical application procedures for the tissues or cells (see Chapter 29).

12.7. Centralised versus devolved management of tissues and cells

Tissues and cells are either delivered directly to the relevant department or operating theatre in an ORHA (i.e. devolved management of tissues and cells) or they are delivered to a single, dedicated location under the direct supervision of an authorised healthcare professional (i.e. centralised management of tissues and cells). The advantage of devolved management is that the relevant department or unit in the ORHA with the appropriate specialist knowledge takes responsibility for the tissues and cells, whereas under centralised management there may be a more uniform approach to biovigilance and traceability and to ensuring compliance with quality and safety requirements for storage, handling and inventory control of the tissues and cells.

Regardless of the model applied for the management of human tissues and cells, all activities associated with receipt, storage, handling and follow-up should be incorporated into the existing quality-management system of the ORHA. The roles and tasks of officially designated persons should be clearly specified in standard operating procedures (SOPs).

12.8. Incoming inspection at the ORHA

When tissues and cells are received by an ORHA, appropriate personnel should verify and record that:

a. the tissues or cells received correspond to what was ordered and to the information in the accompanying documentation, which must be complete and legible;
b. the shipping containers and primary containers are labelled with the required information – including, where appropriate, the Single European Code (SEC) – and that labels are affixed and legible (see Chapter 14). Separate accompanying documents should provide information that is not included in the primary container label;
c. the shipping container and primary container are intact;
d. the specified expiry dates of tissues or cells have not been exceeded;
e. the transport temperature range was monitored or maintained adequately and is acceptable. For tissues or cells that are transported at low temperatures, maintenance of the required transport temperature can be confirmed by data readout from a temperature logger placed in the shipping container or by a residual coolant in the container (e.g. water ice for refrigerated tissues or cells and dry ice for frozen tissues or cells). The supplying TE should be able to provide, on request, a validation report to show that the required temperature can be maintained in the shipping container for a period of time that exceeds the maximum duration of transport.

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Where the delivered tissues or cells do not comply with the above requirements, the ORHA must liaise with the TE to decide the correct course of action, which could include disposal of the tissues and cells or their return to the TE.

Tissue establishments should provide the ORHA and the end-user clinician with documentation of the donor consent, risk assessment and testing, tissue-related information and tissue-processing details, while ensuring that such information does not compromise the confidentiality of the donor. Alternatively, the TE could provide a statement to the effect that the donor and the tissues comply with all the TE's quality and safety standards and legal requirements for donor consent and testing, along with specific information about the characteristics of the tissues and cells required by the end-user clinician.

12.9. Package insert/instructions and temporary storage before use

Once tissues or cells have been distributed by a TE for clinical use, appropriate storage and handling become the responsibility of the ORHA. Instructions should be available in the package insert that accompanies the tissues or cells that describe the appropriate storage conditions and the proper handling procedures to be followed before clinical application. These instructions must be followed precisely by the ORHA.

Tissues and cells are stored under various temperature conditions, depending on their type, method of preservation and packaging. Where a specific storage temperature is necessary from receipt to clinical application, the storage device (refrigerator, freezer, liquid nitrogen storage tank, incubator, etc.) should be regularly maintained and calibrated and should be secure, i.e. with restricted access. It should be dedicated to the storage of healthcare products and cleaned according to a defined protocol and frequency. It should have functional alarms, and emergency back-up storage capacity should be present. Storage procedures should address the steps to be taken if the temperature is outside defined limits or in the event of equipment/power failure. Failure to monitor and maintain controlled temperatures can result in waste of a precious resource and, if tissues or cells are used, serious adverse outcomes due to deterioration in their quality. All records pertaining to storage temperatures should be retained for at least 10 years.

During the storage of tissues and cells at the ORHA before clinical application, they must kept together with their associated documentation or else the documentation must be reliably linked to the tissues or cells and easily accessible. The accompanying documentation should specify the presence of particular additives or reagents that may adversely affect the recipient (e.g. antibiotics, allergens). If there is no package insert accompanying the tissues or cells, they should not be used.

Some EU member states regard short-term storage of tissues or cells at an ORHA as a licensable activity that requires specific authorisation from a Health Authority. Therefore, it is important to be aware of the national legislation in place for the storage of tissues or cells at an ORHA.

12.10. Inspection of the container, documentation and tissues or cells

Upon arrival, the container must be inspected and the accompanying documentation must be reviewed and confirmed to be complete and legible. The label should be checked and compared with the description on the package insert to confirm that the material is indeed what was ordered for the patient and is what is shown on the label. The packaging and the contents should be inspected for any signs of damage during transport. Where temperature during transport and storage at the ORHA is critical, there should be confirmation that the required temperature has been maintained.

In the case of tissues, the graft should be examined once the container has been opened to confirm that the anatomical characteristics are as shown on the label (e.g. left *versus* right femur, aortic *versus* pulmonary heart valve).

Tissues to be used in surgery should be specified and their use documented in the surgical checklist.

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12.11. Preparation of tissues or cells before use

Instructions for opening the container or package, and any required manipulation/reconstitution (e.g. thawing, washing, rehydration), as well as information on expiry dates after opening/manipulation and the presence of any potentially harmful residues or reagents that may adversely affect the recipient (e.g. antibiotics, ethylene oxide), must be provided on the label or in the documentation accompanying the tissues or cells.

The handling instructions provided by the TE for the preparation of tissues and cells for human application should be followed precisely. Any departure from the instructions provided by the TE is at the discretion of the clinical user, who must take full responsibility for any adverse outcome resulting from not adhering to the instructions provided by the TE.

12.12. Surplus or unused tissues or cells

Tissues or cells remaining from a clinical procedure must not be used in another patient; any residue should be discarded as clinical or anatomical waste, in accordance with national rules, or returned to the supplying TE for appropriate and lawful disposal. Similarly, a single unit of tissues or cells (e.g. two halves of femoral head delivered in one container) must not be used in more than one separate patient. Activities that are routinely performed to finally prepare tissues and cells just before their clinical application, e.g. shaping of tendons or bone grinding for impaction grafting, are not considered as processing and do not require notification to the RP at the supplying TE.

Tissues or cells provided to one ORHA should not, in general, be sent to another ORHA for clinical application. Within the EU, this would be defined as distribution and it would require specific authorisation. However, such transfer of tissues and cells may be acceptable where the TE manages the process and the quality and safety requirements of the tissues and cells are not in any way compromised.

Tissues or cells that are received and not subsequently used in one department of an ORHA may be sent to a different department or operating theatre in the same ORHA. However, the details of such activity must be specified in an SLA between the different departments. There may be nationally established rules for such circumstances.

The documentation that accompanies the tissues and cells should specify whether they can be returned to the TE if not opened or used, e.g. if the patient is not well enough for surgery or if surgery is cancelled for another reason. Tissue establishments that do accept the return of unused and unopened tissues or cells must be able to confirm that the required storage conditions have been maintained, that the packaging has not been tampered with, and that the quality and safety of the tissues and cells has not been compromised.

12.13. Traceability

Coding and traceability are addressed fully in Chapters 15 and 16. In the EU, ORHAs are required to maintain traceability records from the point of receipt of the tissue until 30 years after clinical use or another final disposal. These records (mandatory in the EU) must include:

a. identification of the supplying TE or procurement centre;
b. identification of the clinician/end user/facility;
c. type of tissues or cells;
d. unique product identification;
e. identification of the recipient;
f. date of application.

Details of the tissues or cells applied should be in the recipient's record and in the logbook of the treatment room or operating theatre where they have been applied. However, these records alone are not adequate to permit rapid tracing of patients who might be at risk from a particular donation or processing

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batch. The ORHA should also have an electronic or paper log where all received, transplanted and discarded tissues or cells are recorded. This should provide a robust two-way audit trail to facilitate rapid identification of tissues and cells in the case of a recall by the TE or the Health Authority, or identification of recipients where the TE has been notified of a serious adverse reaction or serious adverse event that may have implications for one or more recipients treated at the ORHA. Careful consideration should be given to where and how this log will be archived for the required period, and the person(s) responsible for its maintenance and safe storage should be clearly identified and documented.

Some TEs require the ORHA to return a traceability form or card providing details of the recipient for each tissue and cell supplied. A copy of the card should be retained in the recipient's medical record. The details should be sufficient to unambiguously identify the recipient and the applied the tissues and cells: i.e. at least three points of identification for the recipient and a unique identifier (e.g. SEC in the EU) for the tissues and cells. Returning the card does not release the ORHA from its responsibility to maintain the above-mentioned traceability records for 30 years after clinical use or another final disposal. Where cards or forms are returned to the TE, the manner of documentation should adhere to national data-protection regulations and should ensure that confidential information is stored in secure systems and that the recipient's privacy is not compromised in any way.

It is highly recommended that when individuals who have been treated with human tissues or cells are discharged from an ORHA, their discharge documentation should specifically mention this fact. Hence, general practitioners looking after the patient in the longer term will be able to associate unexpected symptoms with possible transmission or other reactions from the tissues or cells applied. Moreover, general practitioners should be advised to report any suspicious or unusual findings to the ORHA.

12.14. Recipient follow-up and clinical outcome registries

Depending on the healthcare system, a routine clinical follow-up of the tissue or cell recipient is performed either by ORHA or by another healthcare organisation. The extent and duration of this routine clinical follow-up should, where possible, be standardised for each tissue and cell product and application.

Clinical follow-up data are to be kept at the ORHA in the recipient's records and may be submitted to national/international clinical outcome registries. If clinical follow-up data are collected, see section 12.6 for discussion of the implications for recipient consent. In some countries there may be legal obligations to collect clinical follow-up data. In addition to registries for which clinical outcome data entry is mandatory, there are registries based on voluntary reporting of clinical data. Some of them are national, others international; some are maintained by scientific or professional associations whereas others are held by Health Authorities.

Clinical outcome registries provide 'real-world' data that may give a more realistic overview of outcomes compared with single-centre studies. While randomised clinical trials are considered to provide the highest level of evidence, it is not always possible to apply the results more generally outside the strict inclusion/exclusion criteria of such studies. Registries fulfil an important role in allowing studies on large data sets that can be used for determining recipient outcomes for a wide range of conditions, evaluating factors that influence clinical outcome or that may increase the risk of adverse events, and for validation of TE protocols and practices [5]. Registries have been established for several tissue and cell products including HPC, medically assisted reproduction and corneal transplantation.

Currently, there are two ongoing EU projects concerning the importance of recipient follow-up as a source of information for both TEs and Health Authorities; namely, EURO-GTP II (*Good Practices for demonstrating safety and quality through recipient follow-up*) and VISTART (*Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation*).

The outputs of these projects will provide tools and assessment criteria for evaluating and verifying through clinical outcome studies the quality and safety of novel tissues and cells processing

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methodologies, or novel clinical indications/applications of consolidated tissue/cell products (such as those described in Part E of this Guide: Tissue/Cell Monographs. Another project, which is part-funded by the EU, aims to establish a European Cornea and Cell Transplant Registry (ECCTR), which builds on the experience and expertise of three major corneal transplant registries in, respectively, The Netherlands, Sweden and the United Kingdom.

12.15. Adverse events and adverse reactions

Vigilance and surveillance (V&S) is addressed in Chapter 16. Effective V&S relies heavily on all healthcare professionals involved, from procurement through to application.

SARs, as defined in EU Commission Directive 2004/23/EC and in section 12.2 above, may be detected during or after procurement in living donors or after application in recipients of procured tissues or cells. As SARs in recipients might result from many diverse factors associated with the clinical procedure or with the recipient's underlying condition, clinicians might not consider the applied tissues or cells to be the cause of or a contributory factor to the SAR. However, there is an obligation, legal in the EU, for ORHAs to report known or suspected SARs to the supplying TE, which then must report to the Health Authority.

Serious adverse events (SAEs) are defined in EU Commission Directive 2004/23/EC as any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to transmission of a communicable disease, to death or to life-threatening, disabling or incapacitating conditions for patients, or which might result in, or prolong, hospitalisation or morbidity. If detected by the ORHA, they must also be reported to the TE and the Health Authority.

Tissue establishments that supply tissues and cells should provide ORHAs with clear instructions on how to report SARs and SAEs, preferably using standardised documentation. In general, any suspected adverse reaction or event should be reported immediately by the ORHA to the TE that supplied the tissues or cells, before it is confirmed or investigated, to enable the TE to take appropriate precautionary actions to prevent harm to other recipients and to involve the TE in the investigation process. The ORHA has a key role in supporting and contributing to the TE's investigation of suspected adverse reactions and events.

12.16. Management of recalls and reviews

There are various reasons why a TE may recall tissues or cells that were distributed to an ORHA. A recall may be related to the receipt of new information on the donor's medical or behavioural history that implies a risk of disease transmission, or it may be related to the discovery of an error in processing or a fault or contaminant in a reagent or solution used in processing. It may be instigated by the TE or required by the Health Authority.

When a TE issues a recall, it will be necessary to trace very quickly all the recipients of the particular batch (or donation) of tissues or cells implicated. The existence of a centralised logbook or electronic database that maintains a two-way audit trail of tissues and cells received, with dates of use or disposal and identification of recipients, will greatly facilitate conducting a recall. In many of the most significant cases of disease transmission arising from tissue and cell transplantation, it has not been possible to trace the fate of some of the tissues supplied for clinical use. This could leave some patients at risk and without appropriate follow-up and treatment. In these situations, centralised management of tissues and cells in the ORHA should facilitate effective action.

A review may also be required as part of an investigation of the safety of particular tissues or cells that have been applied to patients in the past. It may require recalling patients for additional testing or other investigations. Again, maintenance of a two-way audit trail is essential for effective identification of potentially affected patients.

6686 ## 12.17. References

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6702 # Chapter 13. Computerised systems

6703 ## 13.1. Introduction

6704 Computerised systems are playing an ever-increasing part in the management of business operations,
6705 including those related to healthcare. Tissue establishments and donor registries may use a wide range
6706 of computerised systems. These can range from simple stand-alone computer systems that use a software
6707 package to track and trend data to fully integrated systems that control a range of processing steps and
6708 present data that will allow release of tissues and cells for clinical applications. In some cases these
6709 systems are relied upon to record consent and donor identity. Computerised systems may also have a
6710 role in controlling the facility (premises) or ensuring that required environmental conditions, such as
6711 air-pressure differentials or particle counts, are maintained (e.g. a building-management system).
6712 Errors and malfunctions of computer systems can go unnoticed and might have serious
6713 consequences. Changes in software must be managed carefully to ensure that data have not been
6714 corrupted or reorganised in a manner that changes their meaning or impact. A review conducted for the
6715 UK Secretary of State for Health, published in 2010, reported the discovery of a systematic error in the
6716 documentation of wishes of organ donation that had probably occurred in 1999 and which potentially
6717 affected the records of > 900 000 individuals [1].
6718 Computerised systems help to bring efficiency to processes. However, if they record critical
6719 information with an impact on donation, processing and release of tissues and cells, they must be
6720 selected and validated just like any other piece of critical equipment [2].

6721 ## 13.2. Planning the implementation of a computerised system

6722 Figure 13.1 describes the different steps in implementing a computer system. It illustrates the design flow
6723 and documents related to specific phases (life-cycle documentation), together with the division of
6724 responsibilities between supplier/vendor and user. Diagram A reports the models for system software
6725 categories 4-5 and diagram B reports the simplified model for system software category 3 (as in Table
6726 13.1). Before implementation of a computerised system at a tissue establishment, it is advisable that the
6727 user has close contact with their information technology (IT) department, or an IT consultant
6728 independent of any supplier of computerised systems.
6729 The tissue establishment needs to:
6730 a. define the system by generating a written description of the functions that it is designed to carry
6731 out, and all human interactions, i.e. functional and non-functional requirements: user requirements
6732 specifications (URS). The URS will be the basis for subsequent testing and verification of the
6733 developed/supplied system. A list of minimal requirements for the computerised system includes
6734 (but is not limited to):
6735 i. the need to manage calculations and printouts (e.g. reports and labels),
6736 ii. the need for data protection (personal access to the system or parts of the system),
6737 iii. the duration of and options for record storage (in general, 10 years is required for quality-system-
6738 related data and 30 years for traceability-related data in the EU),
6739 iv. backup conditions ensuring future access to stored data,
6740 v. the need to connect with other computerised systems/registries (social security registries,
6741 administrative systems, financial systems),
6742 vi. the need for encryption in case information is transferred over an open network,
6743 vii. the need for CE labelling (in EU) if patient data, or data relevant for diagnosis or treatment of
6744 patients, are to be included in the system,
6745 viii. the need for audit trails (registration of GMP-relevant changes or data deletion);

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b. evaluate the different systems available and choose one that meets the established requirements (though the degree of user-friendliness and maintenance should also be taken into consideration);
c. audit the developer/manufacturer to ensure that they can provide a product that meets regulatory requirements;
d. define roles and responsibilities and their division between the user and developer/supplier/manufacturer with regard to testing, user instructions, maintenance, system improvements and access to source codes.

These steps should ensure that the user has all the necessary information about the system to be purchased and that the IT department or IT consultant has received the relevant technical information. It is recommended that the developer/supplier of the computerised system receives proper information about the surrounding/other systems that have to be linked to the system to be purchased. This course of action also minimises the need for ‘work around’ by the user (which can be a source of error).

The computer system that manages the activities of a tissue establishment usually includes hardware, software, peripheral devices and documentation such as manuals and standard operating procedures (SOPs). For further information, refer to the International Organization for Standardization ISO/IEC 12207, ISO/IEC/IEEE 29148, ISO/IEC 27001:2013 and ISO/IEC 27007:2011 [3, 4, 5, 6].

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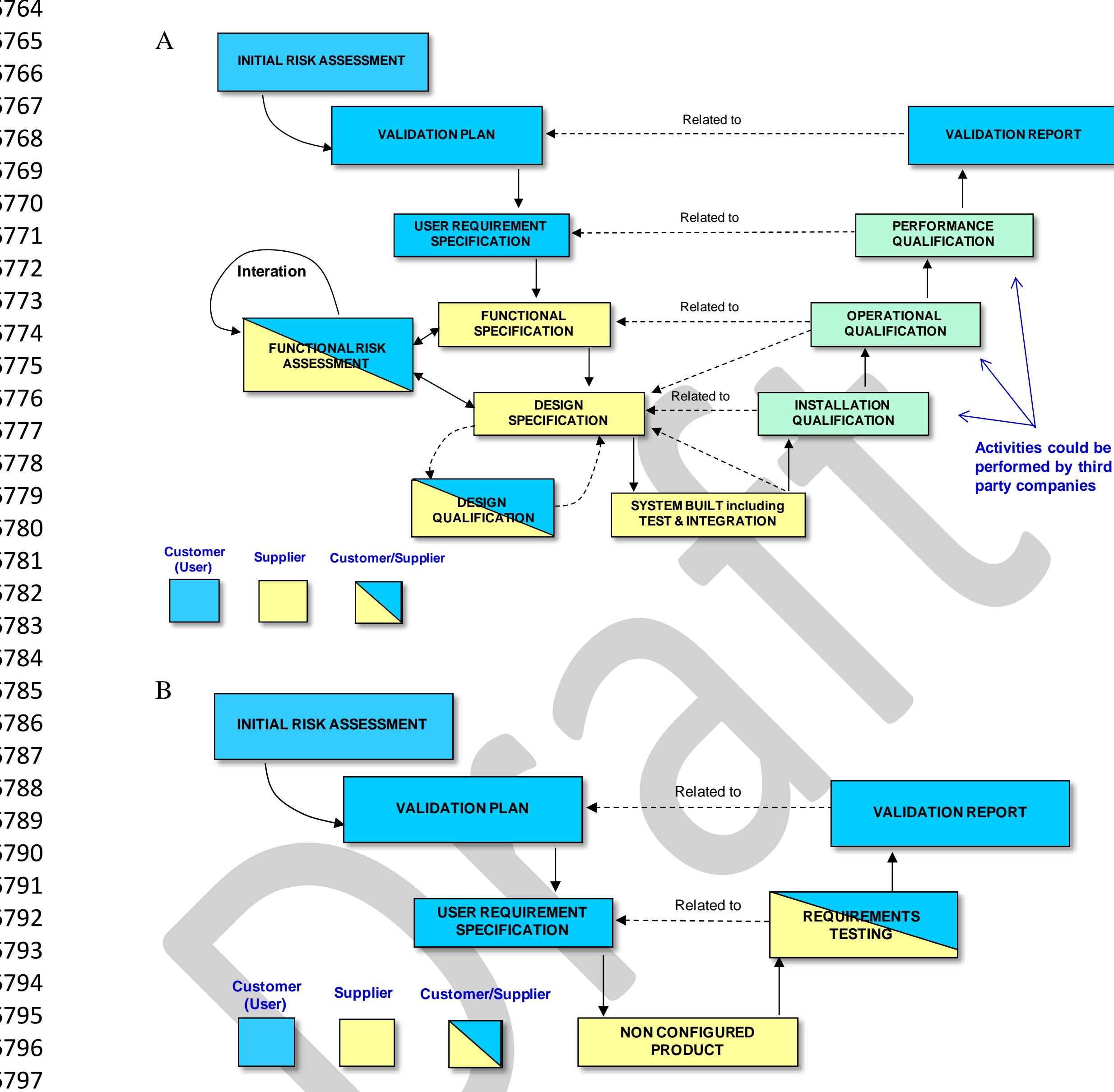

6798 **Figure 13.1. V model diagrams for computer system implementation**

6799 Dashed lines indicate relationships between testing and specification documents. Section A (top) describes system software
6800 categories 4-5; section B describes category 3 (categories as in Table 13.1).

6801 13.3. Verification and testing

6802 The guidance in Chapter 2 on the verification of new equipment should be taken into account. The
6803 verification of computerised systems in a tissue establishment should be incorporated in the general
6804 validation plan of the centre, which should include:
6805 a. the identity of the computerised systems and interfaces that are subject to verification;
6806 b. a brief description of the verification strategies for different categories of computerised systems,
6807 as well as other validation activities;

6808 c. an outline of the protocols and related test procedures for all verification activities of the computer
6809 system (the reporting requirements for documenting the verification exercises and related results
6810 should also be defined);
6811 d. the identity of key personnel and their responsibilities as part of the verification programme.

6812 The level of verification required for computerised systems is dependent on the criticality of the
6813 systems to the quality and safety of the tissues and cells. Therefore, a criticality rating based on a risk
6814 assessment should be applied to all computerised systems in place. The method of verification of these
6815 critical systems depends on the type/category of software used. Table 13.1 gives some examples with
6816 suggested approaches to verification.
6817 Verification should be commensurate with level of risk, intended use and potential implications
6818 of malfunction to quality and safety.
6819 Before verification of a newly installed computerised system can be carried out, a full set of
6820 documentation that is as detailed as necessary to ensure appropriate operation of the system must be in
6821 place. The documentation should include:
6822 a. a detailed specification (inventory) of the hardware, software and peripheral devices, including
6823 their environmental requirements and limitations;
6824 b. diagrams or flowcharts of the system's operations that describe all component interfaces, a
6825 network diagram and all database structures (e.g. file sizes, input and output formats) – if
6826 applicable: i.e. for system software categories 4 and 5;
6827 c. SOPs that describe how the system is used. The user should develop the SOPs, based on the
6828 instructions for use provided by the software developer and the internal procedures of the
6829 establishment. In particular, SOPs should address all manual and automated interactions with the
6830 system, including:
6831 i. routine backup, maintenance and diagnostic procedures, including assignment of responsibilities;
6832 ii. safety leading indicators [5, 6, 7];
6833 iii. 'work-arounds' for system limitations;
6834 iv. procedures for handling errors, including assignment of responsibilities;
6835 v. procedures for handling disasters and contingency planning, including assignment of
6836 responsibilities;
6837 vi. procedures for supervised changes to incorrect data;
6838 vii. procedures for verification of a change;
6839 viii. a training system that includes manuals, documentation and procedures for training.
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6842 **Table 13.1. An approach to verification and control of computerised systems by system category**

Category	Description	Typical examples	Typical approaches
1. Infrastructure software	Software on which applications are built Software used to manage the operating environment	Operating systems Database engines Statistical packages Spreadsheets Network monitoring tools Scheduling tools Document version control tools	Record version number and verify correct installation by following approved installation procedures
3. Non-configured*	Software cannot be configured to suit the specific process, but working parameters can be set to suit the intended use	Firmware-based application* Commercial off-the-shelf software package Instrument software (e.g. software associated with machines used for testing bacteriology or serology, cell counters)	Specify user requirements before selection Risk-based approach to supplier assessment Record version number and verify correct installation Risk-based tests against requirements as dictated by use (for simple systems, regular calibration may substitute for testing) Procedures in place for maintaining compliance and fitness for intended use
4. Configured	Software, often very complex, that can be configured by the user to meet the specific needs of the user's business process; software code is not altered	Management system for donation, processing, storage and distribution of tissues and cells Building management systems (monitoring air pressures in rooms, temperature and/or particles, temperatures of fridges, freezers and incubators) Clinical trial monitoring Note: specific examples of the above system types may contain substantially customised elements	Risk-based approach to supplier assessment Demonstrate that supplier has adequate quality-management system Some life-cycle documentation retained only by supplier (e.g. design specifications) Record version number and verify correct installation Risk-based testing to demonstrate that the application works as designed in a test environment Risk-based testing to demonstrate that the application works as designed in the routine environment Procedures in place for maintaining compliance and fitness for intended use Procedures in place for managing data
5. Custom	Software custom-designed to suit business process	Varies, but may include: Internally or externally developed management systems for donation, processing, storage and distribution of tissues and cells Internally or externally developed process control applications Spreadsheet macro (i.e. database spreadsheet for clinical trial monitoring)	Same as for 'Configured' above, but also: More rigorous supplier assessment, with possible supplier audits Possession of full life-cycle documentation (as indicated in Figure 13.1)
*In GAMP4, firmware applications represented category 2, which is now integrated into category 3. *Source:* modified from ISPE Good Automated Manufacturing Practice (GAMP) 5 [8]			

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6844 Verification documents and the results of tests undertaken and approved by the supplier/vendor
6845 or developer of the system shall be part of the documentation supplied to the user. The user can then
6846 carry out tests according to a predefined and documented test plan [9]. Types of risk to consider include
6847 inadequate design of a system, errors that may occur in use (errors of use or system defects) and loss or
6848 distortion of data [10]. Testing should involve the entire system, and in the manner in which it is expected
6849 to perform routinely in the establishment. Testing may be done by a third party but, in that case, must

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6850 also include personnel from the tissue establishment. The organisation for ownership, system
6851 management, maintenance and support, and the plan for regular internal revisions, should be included
6852 in the quality-management system (see Chapter 2)
6853 The following types of basic testing are examples of what should be conducted initially and when
6854 new versions of the software are installed:
6855 a. Functional testing of components
6856 Initial qualifications are usually carried out by supplier/vendor, who will provide the user with
6857 documentation related to the tests performed. At minimum, documents should include
6858 iv. details of the methods employed to conduct verifications and testing of requisites stated in the
6859 URS document,
6860 v. qualification documents with results of tests (test scripts) for each functionality, including test
6861 procedure, expected result, test result, acceptance criteria, and
6862 vi. conformity statement with relevant signatures.
6863 For traceability and to facilitate quality assurance review and follow-up, it is recommended that
6864 any supporting documentation (e.g. print screens) be included to verify the specific test case.
6865 b. Data migration
6866 The process for data migration should be defined, documented and tested appropriately. This
6867 should ensure full maintenance of traceability, including archiving of data (if necessary).
6868 c. Environmental testing (installation and verification instructions, IVI)
6869 In the actual operating environment, functional tests are carried out to demonstrate that
6870 i. the software systems work appropriately with the hardware;
6871 ii. all applications of the software perform appropriately with the software operating system;
6872 iii. appropriate information passes correctly through system interfaces, including appropriate data
6873 transfer to or from other laboratory and automated (e.g. serology testing, cell counting) systems
6874 (if applicable);
6875 iv. accessories such as barcode scanners perform as expected with the barcodes in use (if
6876 applicable);
6877 v. printed reports are formatted appropriately and correctly;
6878 vi. personnel are trained and use the system correctly;
6879 vii. the system performs appropriately at peak production times and with the maximum number of
6880 concurrent users;
6881 viii. backups restore data in a correct way.

6882 13.4. Change control

6883 In case of changes in the software, the verification status needs to be re-established. If a re-verification
6884 analysis is needed, it should be based on risk assessment and conducted not only for verification of the
6885 individual change, but also to determine the extent and impact of that change on the entire computerised
6886 system.
6887 Changes should be handled following the V diagram in Figure 13.2. Depending on the system, it
6888 may be desirable to have a test version of the computerised system containing the same data (mirrored).
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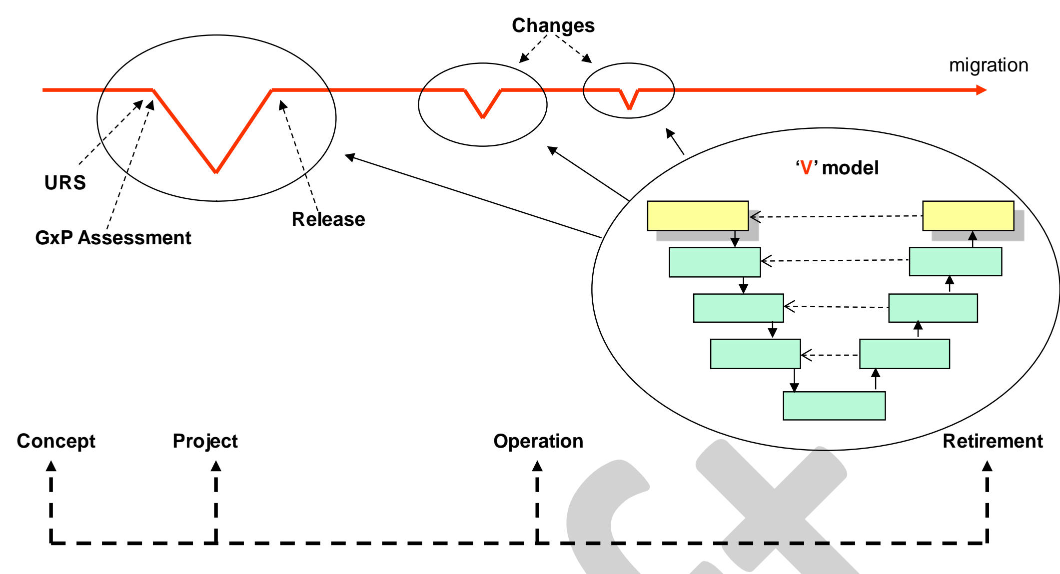

Figure 13.2. Handling of software life-cycle activities

URS: user requirements specifications. GxP: good [specialism] practice. Green: user. Yellow: supplier.
Source: modified from ISPE Good Automated Manufacturing Practice (GAMP) 5 [8].

13.5. Maintenance and scheduled operations

Data should be checked periodically and systematically by qualified IT personnel to identify and remove unwanted data (e.g. duplicate records) and to ensure that data entries are stored accurately and appropriately. Manual entry of critical data requires independent verification by a second authorised person. Please refer to ISO/IEC 14764 for further guidance on maintenance [10].

Security should be maintained by:

a. an adequate change history of the system, for both software and hardware (when necessary);
b. periodically altering electronic passwords (without re-use) and by removing unnecessary or outdated access;
c. creating records of all data changes (i.e. an audit trail), including a retained record of previous data and the reason for the change;
d. appropriate use of programs to detect and remove computer viruses;
e. control of administrative security access to ensure that only authorised personnel can make changes to the software, system configuration and data;
f. regular testing to verify the appropriate integrity and accuracy of backed-up data.

13.6. Quality assurance

The data-processing system should be considered as critical equipment within the quality-assurance programme, which as a minimum should:

a. ensure the ongoing accuracy and completeness of all documentation on equipment, software maintenance and operator training;
b. undertake audits periodically to verify appropriate accomplishment of all performance tests, routine maintenance, change procedures, data-integrity checks, error investigations and operator-competence evaluations.

Appendix 23 contains an example of a checklist that can be used for internal or external audits.

13.7. Industry guidance for verification of computerised systems

The most common industry guide used for verification of computerised systems is that from the International Society for Pharmaceutical Engineering (ISPE) [8]. More specific guidance related to blood and tissues is available from the British Committee for Standards in Haematology [11].

13.8. Regulations governing verification of computerised systems in EU Good Manufacturing Practice

Regulation of computerised systems is well established in the pharmaceutical industry, with EU Good Manufacturing Practices (GMP) [12] acting as the regulatory reference in the EU. Inspectors in the EU also use the Pharmaceutical Inspection Co-operation Scheme Guidance (PIC/S) [13]. The pharmaceutical industry operates on a global scale, so many European companies maintain compliance with the US Food and Drug Administration (FDA) [14]. These regulatory documents can be useful sources of reference for tissue establishments.

If a computerised system replaces a manual operation, there should be no decrease in product quality, process control or quality assurance. There should be no increase in the overall risk of the process [12].

The PIC/S document also lists the critical items that an inspector should consider during inspection and is a valuable tool for tissue establishments since it details the minimum requirements that should be in place [13]. Appendix 23 contains a checklist adapted from ISO/IEC 27007:2011 and the guidance document of the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

13.9. Infrastructure

Infrastructures are necessary in order to guarantee the correct data handling between work stations hosting the computer system and the relevant server(s), and they include but are not limited to communication physical lines (e.g. ethernet), switches and routers. A correct design of a computer system must consider the use of suitable tools (e.g. test suites, servers, version- and configuration-control systems, modelling and architecture tools, communication tools, traceability and behavioural-modelling tools).

13.10. Failure of the system

For computerised systems that support critical processes, provision should be made to ensure continuity of support for those processes in the event of a system breakdown (e.g. a manual or alternative system). The time required to enact alternative arrangements should be based on risk assessment and should be appropriate for the particular system and the business process it supports. These arrangements should be documented and tested adequately [12]. Testing of these alternative systems and their ability to retrieve data should be assessed annually.

13.11. Electronic signature

Records may be signed electronically. According to Annex 11 of EU GMP [12], all electronic signatures are expected to:

a. have the same impact as handwritten signatures within the boundaries of the organisation;
b. be permanently linked to their respective record;
c. include the time and date that they were applied.

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6974 ## 13.12. Data protection

6975 Critical and sensitive data must be protected from unauthorised information modification and from
6976 unauthorised information access/release. Procedures for personal data protection must comply with
6977 national legal requirements or, for EU countries, with the requirements defined in Regulation EU
6978 2016/679 and Directive 2010/45/EU on the Protection of natural persons with regard to the processing of
6979 personal data and on the free movement of such data.

6980 Appropriate technical and organisational measures must be taken to guarantee a level of security
6981 appropriate to the risk, measured against the context and purposes of the processing. The factors which
6982 may be analysed to determine the appropriateness of the measures include degree of data sensitivity, the
6983 risks to data subjects in the event of a breach, and the costs involved in implementing specific types of
6984 security measures. The latter may include:
6985 encryption or pseudonymisation;
6986 measures to ensure the confidentiality, integrity and resilience of processing systems;
6987 methods which enable the timely access to, restoration of or availability of personal data in the event of
6988 an incident;
6989 regular tests and evaluation to ensure that the measures implemented meet their desired objective of
6990 maintaining security of data processing.

6991 All personal data stored in computerised systems must be stored in a secure manner, with access
6992 available only to authorised personnel. The system should ensure data inalterability, and an audit trail
6993 with registration of data access and modifications, including date and identification of personnel
6994 executing modifications. For those applications in which all users should not have identical authority,
6995 some scheme is needed to ensure that the computer system implements the desired authority structure.

6996 ## 13.13. Archiving

6997 Critical data must be archived in a long-term stable medium and placed 'off site' at a location remote
6998 from the hardware, to ensure secure storage. Archived critical data should be checked for accessibility,
6999 readability and integrity. If changes are made to the system (e.g. new computer equipment or software
7000 is installed), then the ability to retrieve archived data must be ensured and tested [12]. Archiving should
7001 be conducted using secure software methods such as databases compliant with ACID (atomicity,
7002 consistency, isolation and durability) requirements, that guarantee data integrity. Files should be stored
7003 in databases, if possible in a time-durable format. Among formats more commonly used are software-
7004 encrypted files and CRC (cyclic redundancy check)-secured files, that require dedicated software to be
7005 managed. As this characteristic may condition future retrieval, an ISO-standardised version of the
7006 Portable Document Format (PDF), called PDF/A [15], has been implemented to ensure document
7007 reproduction using any device in years to come, as the format is independent of hardware and software
7008 platforms.

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7044 **Related document:**

7045 **Appendix 23. Checklist for internal (or external) revision of computerised systems.**
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7047 # Chapter 14. Coding, packaging and labelling

7048 ## 14.1. Introduction

7049 The quality and safety of tissues and cells is dependent not only on the way they are procured or
7050 processed, but on the way they are coded, packaged and labelled before being sent to an organisation
7051 responsible for human application [1, 2]. The World Health Organization (WHO) has published an *aide-*
7052 *mémoire* on the key safety requirements (including storage, packaging and labelling) for essential
7053 minimally processed human cells and tissues for transplantation, and some countries have adopted legal
7054 requirements to ensure that human tissues and cells are appropriately packaged, labelled and coded [3].
7055 These steps are also addressed in the EU tissues and cells legislation. In this chapter, the coding of tissues
7056 and cells, their packaging and labelling requirements are discussed.

7057 ## 14.2. Coding

7058 With increasing movement of tissues and cells across borders, the capacity to uniquely identify them is
7059 essential. This can be achieved by coding that facilitates tracing the tissues and cells from donor to
7060 recipient and vice versa while respecting data protection and confidentiality rules.
7061 Coding started with the development of local coding systems applied in individual tissue
7062 establishments, but in the last two decades there has been significant movement towards the use of
7063 national and international coding standards, building on the longer and more consolidated experience in
7064 blood transfusion.

7065 ### 14.2.1. ISBT 128

7066 ICCBBA (the International Council for Commonality in Blood Banking Automation) manages
7067 ISBT 128 [4, 5], which is the most widely used information standard for medical products of human
7068 origin, including tissues and cells. ICCBBA is a not-for-profit, non-governmental organisation in official
7069 relations with the WHO, and ISBT 128 is endorsed by 21 scientific and professional organisations. The
7070 standard is developed and maintained with input from more than 250 volunteer experts in the fields of
7071 transfusion and transplantation from around the world and provides a structured product terminology
7072 with more than 2 500 defined cell and tissue product codes.

7073 ### 14.2.2. Eurocode

7074 Eurocode International Blood Labelling Systems e.V. (Eurocode IBLS) [6] is a not-for-profit
7075 association under German law. Eurocode IBLS manages the coding standard Eurocode, which is an
7076 ISO15418-listed information standard providing worldwide unique identifiers for labelling blood
7077 products, cells and tissues to enhance security in blood transfusion and cell and tissue transplantation.
7078 Today it is used in Germany and Austria.

7079 ### 14.2.3. Single European Code for tissues and cells

7080 In 2015, the European Commission adopted Directive (EU) 2015/565, amending Directive
7081 2006/86/EC [7] as regards certain technical requirements for the coding of human tissues and cells,
7082 which establishes the EU Coding Platform and the Single European Code. The EU Coding Platform
7083 (https://webgate.ec.europa.eu/eucoding) provides access to the EU Tissue Establishments Compendium,
7084 the EU Tissues and Cells Products Compendium (EUTC) and a code-translator application. The
7085 directive introduces the obligation on tissue establishments to affix a Single European Code (SEC) on

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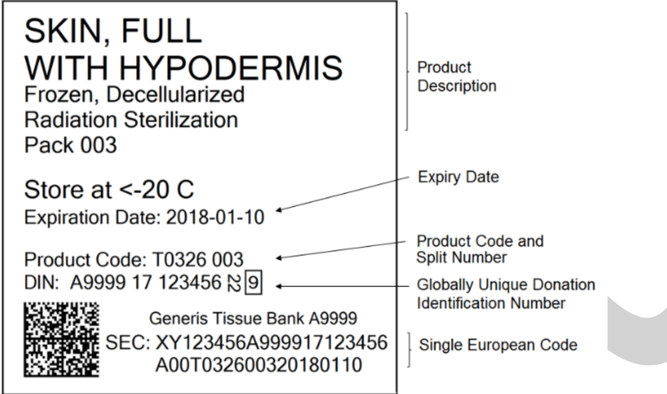
tissues and cells distributed or imported for human application in the EU or exported from the EU [8]. The directive also sets out the requirements for its application (including exceptions) and the general obligations of tissue establishments, competent authorities and the European Commission.

The SEC provides for standardisation across the EU. The permitted product coding systems are ISBT 128, Eurocode and the EUTC (Figure 14.1). The ISBT 128 and Eurocode product descriptions are mapped to the high-level product description codes provided by the EUTC (see § 14.2.3.3).

Label with SEC derived from ISBT 128



Label with SEC derived from Eurocode

Figure 14.1. Examples of finished tissue product labels with the Single European Code

14.2.3.1. Application of the Single European Code

Except for the exemptions described later, application of the SEC [8, 9, 10] by EU tissue establishments is mandatory for all tissues and cells distributed for human application. When tissues and cells are released for circulation (e.g. to other tissue establishments, third parties, manufacturers of

advanced therapy medicinal products), the primary container must include a unique identification number or code and the donation identification sequence (DIS) (see Table 14.1). If the container is too small to include the DIS on the label, the DIS shall be included in the accompanying documentation.

There are some general exemptions to the requirement for application of the SEC code. These include partner donation of reproductive cells, tissues and cells distributed directly for immediate transplantation to the recipient (e.g. HPC), and tissues and cells imported from non-EU countries into the EU in cases of emergency that are authorised directly by the Health Authorities. EU member states may also allow exemptions for tissue and cells other than partner gamete donation, when these tissues and cells remain in the same centre or when tissues and cells that are imported from non-EU countries into the EU remain within the same healthcare facility from importation to application (provided that the healthcare facility is a tissue establishment authorised to import tissues and cells).

Application of the SEC does not preclude additional application of other codes in accordance with the national requirements of EU member states.

Countries already using existing coding systems compatible with the SEC requirements (i.e. ISBT 128, Eurocode) with a standard for barcoding and other forms of machine readability can continue using those systems while incorporating the new legal requirements. There will also be the potential of making the SEC machine-readable in the future. The use of machine-readable barcode labels will ensure the accuracy of records, as manual transcription errors will not occur, and the machine output can easily be entered into electronic databases.

Tissues or cells imported from third countries for distribution in the EU must also be labelled with the SEC (unless the EU member state applies the exemption above). The importing tissue establishment is responsible for the application of the SEC on the product and in the accompanying documentation (double coding/labelling with both the original code and the SEC).

The DIS (see Table 14.1) shall use the tissue establishment number allocated to the importing tissue establishment in the EU Tissue Establishment Compendium. Imported tissues or cells that are already labelled with a globally unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS) shall use this as the unique donation number. If the imported tissues or cells do not carry an identifier from one of these systems, the importing tissue establishment must assign its own unique number. The importing tissue establishment must retain traceability mapping between the identifier they have assigned and the original identification of the imported tissues or cells. Consideration must be given to the possibility that the original identifier may not be unique if products are received from more than one source where the suppliers have used local donation numbering systems; it is quite possible that the same identifier may be used by different suppliers to identify completely different donations. The traceability mapping must therefore include both the original identifier and the supplier identification.

If the imported tissues or cells are already labelled using ISBT 128 or Eurocode, the product code from the original label may be used in the product identification sequence (see Table 14.1) provided that this code is listed in the EU Product Compendium. In all other cases, the importing tissue establishment must assign a product code from one of the three product coding systems (EUTC, ISBT 128 or Eurocode) that is listed in the EU Product Compendium and most accurately describes the imported tissues or cells.

Split numbers carried by imported tissues or cells that do not exceed three alphanumeric characters can be used directly in the SEC. If the imported tissues or cells carry a longer split number, or where no split number is provided, the importing tissue establishment must assign a new split number with a maximum of three alphanumeric characters to ensure uniqueness of the SEC. Particular care needs to be taken where the product code being assigned to the imported tissues or cells is more generic than the original product; for example, bone rings and bone dowels imported with the same donation number where each product is identified by a product code assigned by the supplier and with a split number of 001. The importing tissue establishment applies the SEC using the EUTC of MUSCULOSKELETAL, BONE, SHAPED GRAFT, which means that the two different original product codes have now been mapped to one EUTC code. It is, therefore, no longer possible to use the

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allocated split numbers as this would result in duplication and the importing tissue establishment must assign a new split number and retain records to map back to the original identifiers.

14.2.3.2. Structure of the Single European Code

The SEC is a unique identifier that consists of two elements: a donation identification sequence that indicates the origin of the tissue or cells, and a product identification sequence that describes the type of tissue or cells. Further details are specified in Annex VII to the directive (see Table 14.1).

Table 14.1. Single European Code for tissues and cells

DONATION IDENTIFICATION SEQUENCE			PRODUCT IDENTIFICATION SEQUENCE			
EU TISSUE ESTABLISHMENT CODE		UNIQUE DONATION NUMBER	PRODUCT CODE		SPLIT NUMBER	EXPIRY DATE (YYYYMMDD)
ISO country code	Tissue establishment number		Product Coding System identifier	Product number		
2 alphabetic characters	6 alpha-numeric characters	13 alpha-numeric characters	1 alphabetic character	7 alpha-numeric characters	3 alpha-numeric characters	8 numeric characters'

14.2.3.2.1. Donation identification sequence

The coding system must identify each donation event because donors can potentially donate tissues and cells on several occasions (e.g. an individual may donate gametes and HPC when alive and corneal tissue after death). Each tissue establishment authorised in an EU member state shall use the tissue establishment number allocated in the EU Tissue Establishment Compendium which, in combination with the International Organization for Standardization (ISO) country code, will create the tissue establishment code.

Each tissue establishment shall assign a unique number for the donation based on the donation identification system in place in their country. Donation numbers with fewer than 13 characters will be padded with leading zeros in the SEC. The unique donation number may be created locally by the tissue establishment, centrally (by a Health Authority) or globally as a unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS).

Taken together, these codes will ensure that each donation event will have a unique donation identification number that can be used to label each tissue product. In the case of pooling of tissues and cells, a new donation identification number must be allocated to the final product.

14.2.3.2.2. Product identification sequence

The product identification sequence consists of the assigned product code, a split number (if applicable) and the expiry date of the product (if applicable) in ISO standard format (yyyymmdd). For tissues and cells without a defined expiry date, the expiry date shall be 00000000. The product code includes an identifier of the coding system used ('E' for EUTC, 'A' for ISBT 128 and 'B' for Eurocode) followed by the appropriate product number corresponding to the tissue/cell type.

As explained above, and also taking into account the coding practices used by the EU member states, the SEC provides a flexible solution by allowing tissue establishments to use one of three product coding systems (EUTC, ISBT 128, Eurocode) for which all tissue and cell codes have been included in the EU Tissue and Cell Product Compendium. Tissues and cells in the three product coding systems are mapped to each other to ensure that a tissue or cell product code in the SEC can be 'translated' irrespective of the system used. EUTC provides only the basic nomenclature, but ISBT 128 and Eurocode include more detailed product information (e.g., EUTC may represent a product type such as a tendon, whereas the other two systems may specify whether the tendon is whole, shaped or irradiated).

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EU member states may decide to permit tissue establishments to use only one product coding system (EUTC, ISBT 128 or Eurocode), or more than one in parallel.

The SEC on the label attached to each product will be in eye-readable format and preceded by the abbreviation 'SEC'. The DIS and product identification sequence shall be separated by a single space or as two successive lines. Using ISBT 128, a data structure is available to allow the SEC to be machine-readable.

14.2.3.3. EU Coding Platform

The EU Coding Platform introduced by Directive 2006/86/EC (as amended by Directive 2015/565/EC) is the major tool for implementing the SEC requirements. It is an IT platform hosted by the Commission and it contains the EU Tissue Establishment Compendium and the EU Tissue and Cell Product Compendium [11].

a. The EU Tissue Establishment Compendium is the register of all tissue establishments that are authorised, licensed, designated or accredited by each EU member state's competent authority or authorities; it contains the information about these tissue establishments along with their corresponding tissue establishment codes. The EU Tissue Establishment Compendium is hosted by the European Commission and maintained by the member states' competent authorities. Each competent authority is responsible for the accuracy of the entries for the tissue establishments that they have licensed or authorised and for keeping these entries up to date.
b. The EU Tissue and Cell Product Compendium is the register of all types of tissues and cells circulating in the Union and the respective product codes under the three permitted coding systems (EUTC, ISBT 128 and Eurocode IBLS).

Acknowledging the existence of product coding systems already in use in the EU, Directive 2015/565/EC allows the use of ISBT 128 [12] and Eurocode [13] coding systems, and has put in place bilateral agreements with their managing organisations (i.e. ICCBBA and Eurocode IBLS) to ensure that updated product codes are regularly made available and included in the EU Tissue and Cell Product Compendium.

The EUTC tissue and cell product coding system was developed by the European Commission for tissue establishments not using the other two coding systems. The EUTC covers all types of tissues and cells along with high-level terminology and their corresponding product codes. A mapping of the more detailed ISBT 128 and Eurocode product codes to the generic EUTC codes is also provided on the EU Coding Platform.

Each tissue and cell product shall be assigned a specific code, which identifies and describes that product. The information in the SEC can be decoded by the code-translator application in the EU Coding Platform to obtain text that describes the tissues or cells and their origin.

These tools are publicly available and free of charge. Therefore, the EU tissues and cells product coding system used by EU member states may also be used by other countries. Further information on the SEC and its application can be found on the European Commission's website [8, 9, 10].

14.3. Packaging and labelling

Packaging of tissues and cells has an important role during all procedures, starting from procurement, through the processing and storage steps, to distribution and human application. Adequate packaging minimises the risk of contamination of tissues and cells, protects the persons involved in transportation and aids retention of required characteristics and biological functions.

Ensuring the traceability of all tissues and cells from the donor to the recipient is a responsibility shared by procurement centres, tissue establishments and organisations responsible for human application. All of these participate and contribute actively to safeguarding, in a continuous manner, the tracking of the tissues and cells through from procurement to human application. Accurate tracking of tissues and cells allows reliable data to be scientifically assessed for potential risks to the donor, to the

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7241 procurement and processing operations, and to the storage, transport and clinical use of donated material.
7242 Traceability is addressed in depth in Chapter 15. An essential aspect of ensuring accurate traceability is
7243 clear and complete labelling of tissues and cells at all stages. The system of identification for donors and
7244 recipients must be aligned with the packaging and labelling system of tissues and cells in such a way
7245 that a connection between tissues and cells, the source and the recipients exists at all times.
7246 Labels must be attached to packaging that has been validated to demonstrate that it maintains the
7247 required properties of the tissues and cells and ensures integrity. This part of the chapter addresses good
7248 practice in packaging and labelling at all stages from donation to implantation.

7249 **14.3.1. General concepts**

7250 Packaging and labelling operations must be considered an integral part of the activities of
7251 procurement organisations and tissue establishments. They must be included in the training of personnel
7252 and specified in all relevant procedures. Although this chapter establishes specific recommendations for
7253 packaging and labelling for the procurement and processing phases, they should equally apply to
7254 intermediate phases, such as in-process steps, in which all materials, containers, equipment and
7255 unfinished tissues and cells must be adequately identified at all times. In addition, tissues and cells
7256 procured or processed for research purposes should be clearly identified as such on their packages and
7257 labels (e.g., 'FOR RESEARCH USE ONLY' or 'NOT FOR CLINICAL USE').
7258 There should be written procedures describing the receipt, identification, quarantine, sampling,
7259 examination, testing and release of packaging and labelling materials, as well as the handling of such
7260 materials.
7261 Premises and procedures for the packaging and labelling of tissues and cells must be designed to
7262 prevent cross-contamination or mix-ups. Simultaneous operations should be avoided or, where
7263 unavoidable, adequate additional safeguards should be put in place.
7264 Primary packaging and labelling of tissues or cells must be done in an environment specified in
7265 standard operating procedures (SOPs).
7266 For EU member states, the requirements for packaging and labelling of tissues and cells are
7267 detailed in Annex IV of Directive 2006/17/EC, Annex II of Commission Directive 2006/86/EC and
7268 Commission Directive (EU) 2015/565.

7269 **14.3.2. Packaging of tissues and cells**

7270 Packaging includes all operations, including primary and secondary packaging, which procured
7271 or processed tissues and cells undergo from the start, during processing or as final packaging. Packaging
7272 aims to protect tissues and cells, and to present them to the operator (in initial or in-process packaging)
7273 or to the clinical end user (in final packaging) in a suitable manner. The type of substance of human
7274 origin and its intended use will determine the requirements needed to carry out a packaging operation
7275 in a safe manner.
7276 Special consideration must be given to the primary packaging that will be in direct contact with
7277 tissues and cells. Containers intended to be used as primary packaging should be submitted to visual
7278 inspection before use and, if single-use containers are unavailable, the need for applying an adequate
7279 cleaning process should be assessed along with suitable sterilisation methods such as irradiation or
7280 autoclaving of materials and containers. If the cells/tissues are stored in liquid nitrogen, they must be
7281 double-bagged to prevent cross-contamination during storage [14]. The packaging materials should be
7282 stored in a clean area. In this case, the materials and the conditions under which packaging takes place
7283 must be carefully specified, assessed and approved before use. Processing facilities must establish and
7284 document validated packaging protocols.
7285 Packaging must ensure the integrity and maintain the sterility of the contents of the primary
7286 container. Storage containers must be appropriate for the type of tissue or cells, the temperature and
7287 method of storage, and the intended application. They must withstand sterilisation (where this is to be
7288 applied), not produce toxic residues during storage and be adequately robust to remain intact when

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7289 handled during transport. Each tissue or cell container must be examined visually for damage or
7290 evidence of contamination before distribution for clinical use and by the end user.

7291 **14.3.3. Labelling of tissues and cells**

7292 Written procedures must be established and followed to ensure correct labelling. Each labelling
7293 phase for all tissues or cells must be documented. Tissues and cells must be labelled during all phases
7294 of procurement, processing, storage and distribution. Labelling must be clear, legible, indelible and
7295 unique.

7296 Before labelling a unit of donated or processed tissues and cells, the container must be inspected
7297 for evidence of impurities, defects, broken seals or contamination that could compromise the quality,
7298 integrity or safety of the product.

7299 Labels attached to the containers should identify and describe the contents. The description should
7300 characterise the tissues and cells, and reflect key aspects of their maintenance and use. Standard
7301 nomenclature and standard international units of measurement must be used to describe the tissues and
7302 cells, and the processing they have undergone (see §14.2 on coding).

7303 Identification should provide information on traceability that links the tissues and cells to the
7304 tissue establishment of origin and, ultimately, the donor. When tissues or cells are to be distributed
7305 internationally, language barriers should be considered, and information translated or coded to ensure
7306 understanding.

7307 For autologous or directed donations, the name or identifier of the intended recipient must be
7308 included in the label. Further guidance on traceability is provided in Chapter 15.

7309 The production of labels must be controlled. When applicable, reconciliation of labels that have
7310 been edited, used or returned/rejected must be undertaken according to written procedures. All excess
7311 labels containing quality or traceability information must be destroyed or maintained in a secure manner,
7312 when necessary, to prevent mix-ups. Obsolete, unused labels must be destroyed according to written
7313 procedures.

7314 It is highly recommended to undertake labelling and packaging simultaneously, in a continuous
7315 process, to reduce the risk of mix-ups or cross-contamination. Before application to the container,
7316 printed labels must be carefully examined to ensure that the information they contain conforms to the
7317 corresponding tissues or cells. The results of this examination should be documented at identified critical
7318 stages. Labels must be designed to adhere firmly to the container under all anticipated storage and
7319 transport conditions. The label applied must not be removed, altered or obscured. A sufficient area of
7320 the container must remain uncovered to permit inspection of the contents, whenever possible.

7321 Where additional labels are applied to packaging, an automated verification step to ensure the
7322 correct match between container label and package label is recommended.

7323 For processing of batches that include large numbers of individual final units, a representative
7324 printed label should be included in the processing batch record.

7325 In the European Union, the requirements for final labelling of tissues and cells for distribution are
7326 detailed in Annex II.E of Directive 2006/86/EC. Following the adoption of the Directive (EU) 2015/565,
7327 the label also needs to include the SEC and, for imported tissues and cells, the country of procurement
7328 and the exporting country (if different from the procurement country).

7329 14.4. Sample and documentation labelling

7330 All key cell and tissue samples for testing or archiving and all related documents must be labelled in a
7331 legible, indelible and unique manner that ensures traceability to the donor and the associated donations.
7332 A record of the time and place the sample was taken must be included on the label or in accompanying
7333 documentation.

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14.5. Management of packaging and labelling materials

Selected packaging material must be able to withstand the requirements of the storage temperature (ambient temperature, refrigeration, freezing, cryopreservation) and sterilisation procedure (if this is to be applied) needed to preserve the required characteristics of the tissues or cells and, if applicable, biological function. Additionally, the shipping container must be able to maintain this environment for an appropriate amount of time during transport. Primary packaging and transport containers used for tissues and cells should be validated for this purpose, and they must be suitable for use with human materials (see Chapter 2). Selection of packaging, or a combination of packaging systems, should result in a sealed environment that prevents leaks.

As a general rule, labels should be machine-printed for clarity. They should be printed with ink that does not run or otherwise become unreadable when exposed to water or other liquids. Labels must maintain integrity and remain attached to primary packages and transport containers at the storage temperatures.

All printed labels for primary packaging, secondary packaging and for documentation intended to accompany the tissue or cell product should be stored in access-controlled areas.

Management of packaging and labelling materials must include the following elements:

a. there must be written specifications for all packages and labels used for tissues and cells;
b. there must be documented procedures describing the receipt, identification, quarantine, sampling, examination, testing, release and handling of both packaging and labelling materials;
c. a version control system should be in place to guarantee use of the current approved version. If a change of version occurs with regard to labels, inserts or packages, the actions needed to ensure that only the latest version is attached to the tissue or cells should be described in a written manner;
d. the suitability of packaging material, containers and labels for their intended purpose must be documented.

14.6. Primary packaging and labelling for procurement operations

'Primary packaging' refers to the materials that will come into direct contact with the tissues and cells and are, therefore, considered to be 'critical'. The selected materials should not leach harmful chemicals, they should be capable of being sterilised by a safe method (if required) and they should be sealable, leakproof and traceable.

After procurement, all tissues and cells must be packaged in a manner that minimises the risk of contamination and must be stored at temperatures that preserve the required characteristics and biological functions of the tissues and cells.

Packaging must also prevent contamination through exposure to those persons responsible for handling and transportation of the tissues and cells.

Procured tissue must be inspected and recognised appropriately before packaging and labelling to avoid mix-ups. Each tissue must be packed separately in sterile packaging as soon as possible after recovery. Double or triple wrapping may be necessary, depending on the tissue-specific requirements. Musculoskeletal tissues and skin may be packed in sterile, transparent polymer foil (though additional cotton wrapping can be used) or in containers with or without transport medium. Corneas must be placed in sterile transparent containers with medium, whereas heart-for-heart valves, amniotic membrane, skin, or cartilage for cell cultures must be packed in sterile containers with transport medium. Whole eyes must be stored separately in moist chambers. Composition of the transport medium for a particular type of tissue must maintain the biological properties of tissues and may include antibiotics and antimycotics validated by type and concentration.

Procured cell products are mostly packaged in disposable bags. These bags are also double wrapped before the product is transported. Reproductive tissues and cells are mainly packed and transported in straws or tubes, either in culture medium or cryopreserved.

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A unique identification number or code shall be allocated to the donation and to donated tissues and cells during procurement, or at the end of the recovery process, to ensure appropriate identification of the donor and traceability of all donated material.

The minimum information that should be present in a primary label is described in Table 14.2. If any of the information listed in Table 14.2 cannot be included on the primary package label, it must be provided in accompanying documentation inside the transport container. Small containers, such as straws, must be labelled at least with a unique identification number or code (e.g., treatment code, donation number, or similar) and this identifier must be provided on the accompanying documentation. Table 14.2 lists the required information that should be provided either on the label or in accompanying documentation.

14.7. Secondary packaging and labelling for procurement operations

If secondary packaging is used after procurement, it should adhere to the same requirements as those established for primary packaging. If labels with all the required information are not attached to the primary packaging they should be attached to the secondary packaging, which should be closed and sealed, ensuring that any unique identification number on the primary label is present on the label for the secondary pack and on accompanying documentation.

14.8. Outer container packaging and labelling for procurement operations

Packaged tissues and cells must be shipped in a container that is suitable for the transport of biological materials and maintains the safety and quality of the tissues or cells. Temperature conditions between recovery and processing must be appropriate for the type of tissue or cell to preserve the required characteristics and biological functions (i.e. temperature and duration of transport to the tissue establishment where the tissue processing will take place). The container must be closed fully with a tamper-evident seal and not opened until the procured tissues or cells are received by the tissue establishment.

When tissues or cells are shipped from the procurement site to the tissue establishment, the transport container must be labelled with the information described in Table 14.3.

14.9. Procurement package insert

It is recommended that the documentation accompanying the procured tissues or cells indicates, where applicable, that they are in a state of 'quarantine' to ensure that it is clear that a final review regarding their release for distribution and use has not been completed. See Chapter 6 for full guidance on the requirements for procurement documentation.

14.10. Packaging and labelling during processing

Labelling of unfinished tissues or cells during intermediate phases of processing shall be applied to all packaging materials and containers to assure identification at all times.

14.11. Packaging and labelling for finished tissues and cells

14.11.1. Primary packaging and labelling for finished tissues and cells

Primary packaging and labelling refers to the materials that will come into direct contact with tissues and cells, and the requirements in this regard are described in section 14.6, with a special focus on the radiation-resistance of packaging material for tissue that will be sterilised by irradiation. The expiry date

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7421 will be determined not only by the properties of the tissues and cells but also by the integrity and stability
7422 of the packaging and labelling materials, among other factors.
7423 Packaging and labelling procedures shall be done to prevent cross-contamination or mix-ups.
7424 Simultaneous operations should be avoided or adequate measures should be taken to ensure that no
7425 cross-contamination or mix-ups occur [15].
7426 Facilities where packaging or labelling operations have taken place should be checked before
7427 starting any other operation to guarantee that all previous materials have been removed.
7428 Printed labels should be examined carefully to ensure that the information contained conforms to
7429 the corresponding tissues or cells. Results of this examination should be documented. A printed label,
7430 representative of those used, should be included in the processing records.
7431 Unused and already printed labels must be destroyed according to written procedures.
7432 The information that needs to be on the primary package label of the finished product is detailed
7433 in Table 14.2.
7434 If the primary container is too small to host a label with all the required information (as may be
7435 the case with, e.g., gametes and embryos), the minimum information on the primary container needs to
7436 be a unique identification number or code. This unique identification number or code and the other
7437 required information must be included in an accompanying document.
7438 The additional information that must be provided either on the label or in accompanying
7439 documentation is described in Table 14.2.
7440

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7441 **Table 14.2. Labelling of the primary container**

Basic requirements
As a minimum, the primary container must include a unique donation identification number or code.
*The information listed in this table in **bold print** must be included on the label if space permits or, if there is insufficient space on the primary container label, the information shown in **bold print** must be included on a separate sheet accompanying the primary container.*
Information listed in normal print must be included either on the label or in accompanying documentation. Accompanying sheets must include the unique donation identification number or code specified on the primary container.

Labelling of procured tissues and cells

- **unique donation number or code**
- **type of tissues or cells**
- **date (and, where possible, time) of procurement**
- identification of the procurement organisation

Any accompanying tissue or blood samples for testing must be accurately labelled to ensure identification with the donor and must include a record of the time and place at which the specimens were taken.

Labelling of tissues and cells from a tissue establishment released for circulation to another operator for further processing

- **unique donation number or code and, for tissue establishments in the EU, the donation identification sequence (DIS) from the Single European Code (SEC); for tissues or cells imported from outside the EU, the DIS must be applied by the tissue establishment responsible for import**
- **identification of the originating tissue establishment**
- **type of tissues or cells**
- **expiry date and, where relevant, time (in UTC if the tissues or cells are to be shipped to another time zone); if an expiry date has not been defined, the expiry date must be recorded in the SEC as '00000000'**
- date of circulation (in accompanying documentation to avoid having to re-label the primary container)
- biological tests/assessments carried out on the donor and the results
- presence of potential harmful residues (e.g., antibiotics, ethylene oxide)

Final labelling of tissues and cells released for distribution to an organisation responsible for human application

- **unique donation number or code and, for tissue establishments in the EU, the SEC; for tissues or cells imported from outside the EU, the SEC must be applied by the tissue establishment responsible for import**
- **types of tissues or cells and lot or batch number where applicable**
- **expiry date and, where relevant, time (in UTC if the tissues or cells are to be shipped to another time zone); if an expiry date has not been defined, the expiry date must be recorded in the SEC as '00000000'**
- description (definition) and, if relevant, dimensions/volume of the tissue or cell product
- date of distribution (in accompanying documentation to avoid having to re-label the primary container)
- biological tests/assessments carried out on the donor and the results
- presence of potential harmful residues (e.g., antibiotics, ethylene oxide, etc.)
- morphological and functional data, where relevant
- a statement that the tissues or cells are suitable for human application according to relevant medical selection criteria and testing for markers of transmissible disease
- a statement limiting use of the tissues or cells to specific health professionals
- a statement, as applicable, that the tissues or cells may not be sterilised or re-sterilised
- a statement that it is the responsibility of the organisation responsible for human application to maintain the tissues or cells according to specified storage conditions and to follow instructions for opening the container, package and, where relevant, any required manipulation/reconstruction
- instructions for reporting serious adverse reactions and/or events

Information to be included on all labels

- **for autologous donations, the label must state 'FOR AUTOLOGOUS USE ONLY'**
- **for directed donations, the label must identify the intended recipient**
- **when tissues or cells are from a donor known to be positive for a relevant infectious disease marker, the warning 'BIOLOGICAL HAZARD' must be included**
- for imported tissues or cells, the country of procurement and, if different from the country of procurement, the exporting country
- nature of additives (if used)
- storage conditions required to maintain the quality and safety of the tissues or cells
- instructions for opening the container, package and, where relevant, any required manipulation/reconstitution
- expiry date after opening/manipulation

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14.11.2.Secondary packaging and labelling for finished tissues and cells

'Secondary packaging' and labelling refers to materials that are not intended to come into direct contact with the tissues and cells. Special consideration must be given when primary and secondary packaging and labelling are designed to be kept together until the moment of use. If secondary packaging is not sterile, it should be clarified in the package instructions that the outside of the primary package is also not sterile and should not be placed within the sterile field during clinical application.

14.11.3.Outer container packaging and labelling for finished tissues and cells

When tissues or cells are shipped for distribution, every transport container must be guaranteed to maintain the conditions needed for the specific tissue or cell type. Containers must provide adequate protection against deterioration or contamination of tissues and cells that may occur during storage and transportation. Containers should be cleaned before use to ensure that they are suitable for their intended use. These containers should not alter the quality, safety or efficacy of the tissues or cells. Records should be maintained for each shipment of labels and packaging materials showing receipt, examination or testing, and whether accepted or rejected. For transport, the shipping container must be labelled with all the same information as specified in Table 14.3.

Table 14.3. External labelling of the shipping container

For transfer of procured tissues or cells from the procurement organisation to a tissue establishment • identification of the originating procurement organisation, including name, address and telephone number of a contact person • identification of the tissue establishment destination, including name, address and telephone number of a contact person
For transfer of tissues or cells from a tissue establishment to another operator for further processing • identification of the originating tissue establishment, including name, address and telephone number of a contact person • identification of the other operator destination, including name, address and telephone number of a contact person
For transfer of finished tissues or cells from a tissue establishment to an organisation responsible for human application • identification of the originating tissue establishment, including name, address and telephone number of a contact person • identification of the organisation responsible for human application destination including name, address and telephone number of a contact person
Information to be included on all shipping labels • a statement that the package contains 'HUMAN TISSUES/CELLS' and the warning 'HANDLE WITH CARE' • where living cells are essential for successful human application, the warning 'DO NOT IRRADIATE' must be added • when tissues or cells are from a donor known to be positive for a relevant infectious disease marker, the warning 'BIOLOGICAL HAZARD' must be added • date and time at the start of shipping • shipping conditions relevant to the quality and safety of the tissues or cells (e.g. 'DO NOT DELAY', 'KEEP COOL', 'KEEP IN UPRIGHT POSITION', 'DO NOT FREEZE') • when shipping by air, it is mandatory under International Air Transport Association (IATA) regulations that an IATA time- and temperature-sensitive label is attached to the outside of the shipping container. The lower half of the label must indicate the permitted external temperature range in degrees Celsius (see §14.12)

14.11.4.Package insert for finished tissues and cells

A 'package insert' refers to the supplementary information associated with tissues and cells that cannot be placed on labels. Critical information for the clinical user must be provided.

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7463 ## 14.12. Customs clearance

7464 For clearance of customs, all tissues and cells crossing borders require a clear description of the content
7465 of the consignment, its destination and intended use. The paperwork sent with the consignment should
7466 include the World Customs Organization Tariff Number for Human Tissue for Transplantation, which at
7467 present is 30029010, but there is a request that this should be altered to base code 082 with subcodes. It
7468 is important that the transport of frozen or cryopreserved products packed in dry ice or stored in a dry-
7469 shipper, must not be delayed at border crossings. If the goods are being transported by air, packages
7470 must be labelled with the appropriate International Air Transport Association (IATA) codes: UN1845 for
7471 dry ice or UN1977 for liquid nitrogen in a dry-shipper, and UN3373 for shipment of biological substances
7472 by air [16, 17]. Therefore, it may be expedient for the importer to inform customs of a prospective
7473 consignment, and any enquiries by customs should always be answered promptly (see Chapter 10). For
7474 tissue or cell transport, the agreement with the shipping tissue establishment should define
7475 responsibilities for meeting the cost of transport and storage under appropriate conditions at a receiving
7476 facility for any items that may be detained pending customs enquiries.

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Chapter 15. Traceability

15.1. Introduction

Clinical application of tissues and cells brings great benefits for patients. There are, however, rare (but important) risks associated with such clinical use, including graft/application failure, donor-transmitted infections, malignancies and genetic conditions. The concept of traceability is the means to link a donor with recipients, or with offspring born through medically assisted reproduction (MAR), and all information about the transferred tissues and cells from donation to clinical outcome and follow-up.

Traceability means the ability to locate and identify the tissue/cell during any step from procurement, through processing, testing and storage, to distribution to the recipient or disposal, which also implies the ability to identify the donor and the tissue establishment receiving, processing or storing the tissue/cells, and the ability to identify the clinicians at the medical facility applying the tissue/cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues/cells [1].

The increased transportation of grafts across national boundaries has made traceability difficult and sometimes impossible [1]. It is therefore essential to facilitate rapid action to prevent harm when links in the safety and quality chain are found to have been compromised. Apart from quality and safety, traceability is also crucial for ethical reasons, as it allows legitimate donation with proper consent to be verified for every tissue or cell product.

The system of traceability is inseparable from, and in practice dependent on, the coding system (see Chapter 14). Effective traceability and biovigilance in the global context depend upon the use of globally unique identification for all donated biologic products [2].

The need to comply with traceability requirements should not compromise the need to guarantee anonymity between donor and recipient (or newborn), depending on the type of donation and the national legislation enforced. Records should be kept by the entities involved in the donation, procurement, processing, storage, distribution and application of tissues and cells to ensure compliance with safety requirements, but records should never allow the disclosure of confidential information to unauthorised persons.

Human error, equipment failure, use of inadequate written procedures or new risks that cannot be predicted may affect quality, safety or effective use of tissues and cells at any stage, potentially increasing the risk to recipients and offspring. In the case of deceased donors, procurement teams are provided with a medical history at short notice, and additional information about the donor at a later stage may have implications for the safety and quality of tissues procured from those donors. Use of defective equipment, poor-quality consumables, contaminated solutions or defective testing kits may only come to light after the tissues and cells have been processed and transplanted. This means that traceability, from donation through to end use, is essential to determine which tissues or cells could potentially be affected by additional information or adverse incidents. For MAR, traceability does not stop when the tissues and cells reach the recipient. The health of the children born as a result of MAR treatment must be followed up, so that data on children's health and follow-up of pregnancies are included in the chain of traceability.

Tissue establishments play a special role in assuring traceability, collecting the data that guarantee the ability to locate and recall tissues and cells or inform the applying clinicians and recipients, once the establishment becomes aware of information that may have implications for their quality and safety. Tissue establishments are responsible for communication with other entities, such as organ transplant units, and other tissue establishments (including MAR centres) involved in the procurement/collection or processing of additional tissues and cells, or cells from shared donors.

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The time interval between detecting risks to the quality and safety of tissues and cells, and preventing them from being used in patients, has been referred to as the 'traceability window period' [3]. Recalls can be due to inappropriate evaluation of donors, positive serology tests in the donor, contamination of tissues or cells, infection in recipients of other tissues donated by an individual donor and other risks introduced during the processing or storage of tissues or cells. The increasing global circulation of tissues and cells for clinical use, the fact that several tissue products can originate from one donor who may also donate organs, or that many children may be born from one sperm donor, and the existence of international markets for equipment, consumables and additives all add to the need for robust systems of traceability.

Records and procedures required to maintain traceability must be kept long after the clinical use of products (see §15.2.e), allowing personnel to track and trace all steps associated with the tissues and cells long after their clinical application, making adequate biovigilance and follow-up procedures possible. Traceability underpins biovigilance (see Chapter 16). Within each tissue establishment, investigation of adverse events and adverse reactions, and of deviations from standard procedures, can be carried out only if a system of traceability is in place. Many establishments share practices and standards, and effective investigations can help to improve them. Hence, in addition to biovigilance, ongoing quality improvement of procedures relating to procurement, processing, donor testing, storage and distribution of tissues and cells also benefits from good systems of traceability.

Traceability requirements are often defined in legal obligations, and may include the ability to report the precise number of units and recipients, for use as denominator data in the evaluation of adverse occurrence frequency at national and international level.

Traceability must encompass all the data associated with the final destination of tissues and cells distributed by third parties, including records of the final distribution of imported and/or exported units.

15.2. How traceability works

Traceability is the 'thread' that joins all the pieces of critical information together, from the moment that a potential donor is identified until the moment when the tissues or cells are applied to the recipient or discarded. This means traceability is a concept allowing (i) tracing of procedures when recipients show any adverse reaction that could be linked with the quality of the tissues or cells distributed, (ii) tracking the fate of recipients and (iii) follow-up of the health of MAR children, and (iv) tracking units associated with incidents detected after distribution or clinical application.

Tissue establishments must ensure that data protection and confidentiality measures are in place, in accordance with the local national data protection laws. Many organ donors are also tissue and cell donors, so it is important that effective links are in place between organ-procurement organisations and tissue establishments.

All records must be legible and indelible, protected from unauthorised amendments, stored securely and readily retrievable. Establishments should conduct regular audits of records to ensure that they are accurate and comprehensive. Good practice requires that amendments to written records are signed and dated. Computer records should be maintained in a validated system (see Chapter 13) and there must be procedures to back up electronic records to prevent loss, corruption and unauthorised access or amendment. Records must be shown to be reliable and a true representation of the events. Records may be handwritten or transferred to another validated system, such as a computer or microfilm. Records should be maintained of equipment and consumables, including the lot numbers and expiry dates of additives, cryoprotectants and packaging materials used during procurement and processing. The tissue establishment should also retain temperature records, analyser printouts and relevant environmental monitoring records for viable and non-viable particles. If tissues and cells have been imported, it is important that tissue establishments ensure that the traceability chain is retained and that the records for traceability (see §15.3) are accessible.

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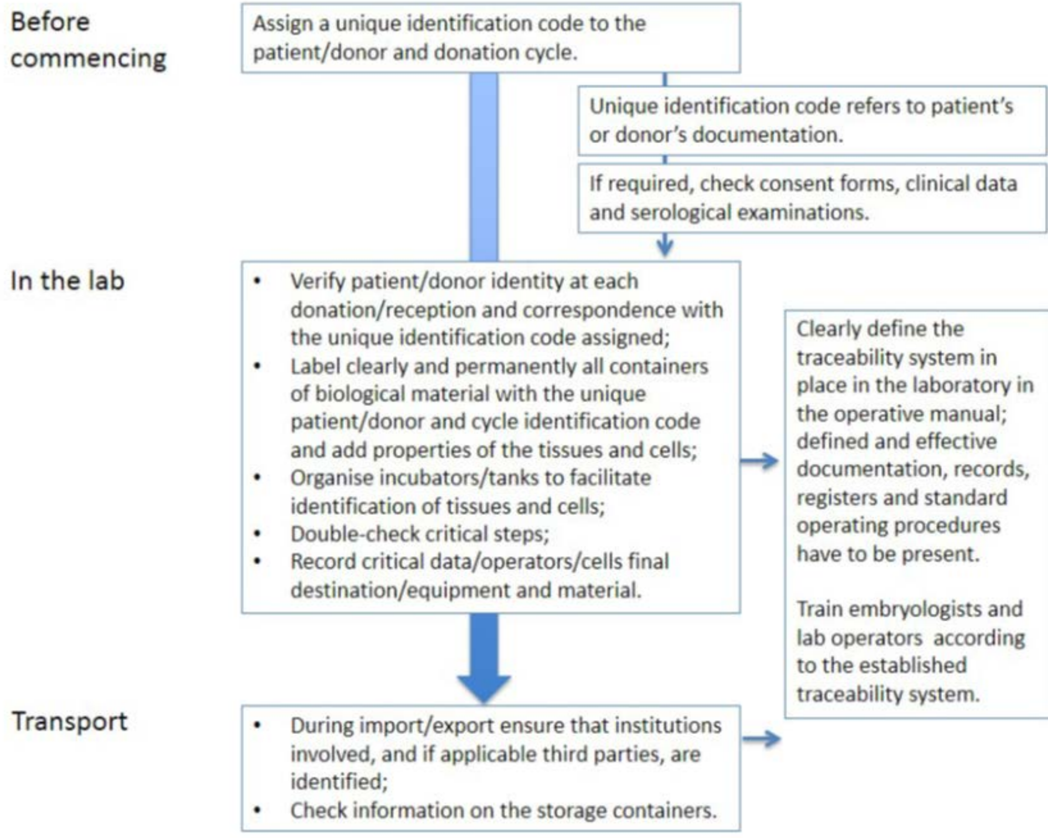

7609 **Figure 15.1. Example of traceability system in medically assisted reproduction**

7610 *Source: reprinted from ESHRE revised guidelines for good practices in IVF laboratories (2015)* [4]

7611

7612 The following are the key requirements of an effective traceability system:

7613 a. Unique identification

7614 At each stage in the pathway, from donor to recipient or child conceived as a result of MAR
7615 treatment, each tissue establishment must have records of the donor, the donation and donation
7616 samples, and must ensure that they are identified and labelled uniquely within their own
7617 organisation (see Figure 15.1 for an example). While uniqueness can be ensured without difficulty
7618 within one organisation, the risks of duplication are increased when tissues, cells, samples or
7619 records move from one organisation to another. For example, duplicate identifiers may result
7620 when samples are sent to a testing laboratory or when tissues or cells are sent to a hospital because
7621 each receiving establishment may assign its own identifier. This risk can be eliminated if a global
7622 standard is used to identify samples or tissue products. Within the European Union (EU), the
7623 Single European Code (SEC) will help to address this need (see Chapter 14). The SEC allows,
7624 within its structure, incorporation of the international coding systems ISBT 128 and Eurocode.

7625 b. Safe transfer of critical information

7626 The traceability trail depends on the accurate transcription of critical identification information.
7627 Manual transcription errors can cause breaks in the traceability trail. Use of electronic transfer of
7628 critical information (bar codes or other machine-readable codes) is recommended. If manual
7629 transcription is used, double checking of data must be implemented. Electronic storage of data –

preferably in well-protected databases for easy and quick access by authorised personnel – is preferred to paper-based information collections.

c. Timeliness
If a risk is identified, it must be possible to rapidly trace all implicated products or all potentially affected recipients and children conceived through MAR. A delay could result in harm to patients or children conceived through MAR. Systems need to be quickly accessible, with efficient links between organisations to reduce the 'traceability window period'.

d. Clarity of responsibilities at interfaces between organisations
To guarantee traceability, tissue establishments should distribute tissues and cells to other tissue establishments, to organisations responsible for human application (ORHA) or to healthcare professionals who have responsibility for clinical application (and not directly to recipients). It is essential that each organisation in the chain clearly understands its responsibilities for traceability. It is notable that in the published high-profile cases of viral transmission during transplantation, hospitals were often not able to trace all recipients [5]. Maintaining traceability is one of the key legal and technical responsibilities of an ORHA (see Chapter 12). Tissue establishments must define responsibilities and procedures prior to the distribution of tissues, cells, gametes or embryos to those organisations.

e. Long-term storage of secure records
For effective reviews, traceability data need to be maintained for long periods of time. For example, in the EU, all information related to traceability must be maintained for 30 years after application or the expiry date of the tissues and cells. Data that are critical to the safety and quality of tissues and cells, including records of equipment used and materials such as consumables coming into contact with those tissues or cells, should be kept so as to ensure access to the data for at least 10 years after clinical use of the product, its expiry date or disposal. Organisations need to consider the impact of the obsolescence of technology and to ensure that records remain quickly accessible. There is a need for regular management review of data storage, with a proactive approach to prevent obsolescence.

f. Traceability provisions
The location of traceability records may change when organisations are closed or merged, or if they cease activities relating to donor selection, donor testing, procurement, processing, distribution or transplantation. In such cases, there must be an effective link between the new location of the data and the previous location, and provision must be made to prevent loss of traceability information, for example by signing contracts with other tissue establishments for taking care of these data in critical situations.

g. Traceability audits
Organisations must include audits of traceability from donor to recipient and vice versa as part of ongoing quality management. The traceability trail may encompass data stored in several organisations.

The EU definition of traceability is provided in the Glossary (Appendix 3) of this Guide.

15.3. Which records must be traceable?

There must be a system of record keeping for all activities associated with tissues and cells. Records should describe donation procurement, donor testing, processing, storage, distribution and end use. Records should include details of equipment used, materials such as consumables that have come into contact with those tissues and cells and the identity of the members of staff who were responsible for all critical activities from procurement until implantation or disposal. These robust systems must ensure secure identification of:

a. the donor and all records associated with the donor and their medical and behavioural history;
b. the donation (tissues or cells procured/collected from the donor);

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7678 c. all records associated with processing, storage and distribution of the final products, and related
7679 events;
7680 d. all samples taken from the donor or from the tissues/cells for the purposes of testing for quality
7681 and safety;
7682 e. the clinical application and recipient(s) of the tissues or cells;
7683 f. the health of the resulting child(ren) and any adverse data on pregnancies (for MAR treatment).

7684 **15.3.1. Records of identification, donor tests and clinical evaluation of the donor**

7685 Besides the information defined in Chapter 4 (on donor evaluation), tissue establishments must
7686 keep in their records at least the following data:
7687 a. donor identity;
7688 b. age, sex, medical and behavioural history of the donor;
7689 c. outcome of physical examination for deceased donors;
7690 d. completed haemodilution algorithm (where applicable);
7691 e. consent/authorisation form;
7692 f. relevant clinical data, laboratory test results and the results of any other tests carried out;
7693 g. for deceased donors, results of the autopsy (if carried out) or preliminary verbal report;
7694 h. for haematopoietic progenitor cell (HPC) donors, the donor's suitability for the chosen recipient
7695 (see Table 15.1 for an example);
7696 i. for unrelated HPC donations, where the organisation responsible for procurement has limited
7697 access to recipient data, the ORHA or the physician should be provided with the relevant donor
7698 data to confirm suitability.
7699

	Donor centre	*National registry*	*BMDW*	*Collection centre*	*Tissue establishment*	*Transplant centre patient*
Activities	Consent Testing Donor follow-up	Listing Donor and patient follow-up	Listing	HPC collection	Product labelling, processing and release	Infusion Patient follow-up
Donor* data	ID code Identity	ID code Identity of National Registry donors only	ID code only	ID code Identity	ID code Product code (e.g., SEC)	ID code only
Patient* data	ID code Identity	ID code Identity	NA	ID code Identity	ID code Identity	ID code Identity

7700 **Table 15.1. Traceability of unrelated HPC donor and recipient data**
7701 *Anonymous contact between patient and donor allowed post transplantation only through Registry.
7702 BMDW: Bone Marrow Donors Worldwide.
7703 The identity and privacy of all patients and donors are protected throughout the process of HPC donation and transplantation
7704 (Identity=name).
7705
7706 In addition, the donor testing records must be accessible at the laboratory (in-house or at a
7707 contracted laboratory) and contain at least:
7708 a. date and time donor blood samples were taken;
7709 b. date of receipt of the blood sample at the testing facility;
7710 c. record of each test kit used to test donor blood sample (i.e. manufacturer, lot number, expiry date);

7711 d. results of donor testing, including repeat testing (if applicable).

7712 Accessibility authorisations and the responsibilities associated with record keeping and reporting,
7713 of both tissue establishments and testing laboratories, should be properly defined through a technical
7714 and legal written agreement (for technical agreements with testing laboratories, see §5.4).

7715 **15.3.2. Records of procurement of tissues and cells**

7716 Besides the information defined in Chapter 6 (on procurement), the organisation undertaking
7717 procurement should produce procurement reports and provide them to the tissue establishment. The
7718 procurement report should contain at least:
7719 a. the identification data of the tissue establishment receiving the tissues or cells;
7720 b. donor identification data (including how and by whom the donor was identified);
7721 c. description and identification of procured tissues and cells (including samples for testing);
7722 d. identification of the person who was responsible for the procurement session, including his/her
7723 signature;
7724 e. date, time (start and end, if relevant) and location of the procurement and standard operating
7725 procedure used;
7726 f. description of the physical area where procurement took place, including environmental
7727 conditions at the procurement site (where necessary);
7728 g. for deceased donors, storage conditions of the deceased donor, i.e. refrigerated (or not) and time
7729 of start and end of refrigeration;
7730 h. manufacturers and lot numbers of reagents and transport solutions used;
7731 i. any incidents that occurred during procurement.

7732 **15.3.3. Records of processing of tissues and cells**

7733 Besides the information defined in Chapter 8 (on processing) and Chapter 9 (on storage and
7734 release), the organisation undertaking processing should keep at least the following records:
7735 a. tissues and cells received and evaluation of their suitability;
7736 b. standard operating procedures used to process the tissues and cells;
7737 c. equipment used during processing;
7738 d. records of consumables used during processing (manufacturer, lot number, storage conditions of
7739 consumables – if appropriate – and expiry date);
7740 e. records of sterilisation or decontamination, if applicable;
7741 f. records of cryopreservation and freezing protocols, if applicable;
7742 g. records of environmental monitoring (temperature monitoring, microbial monitoring and particle
7743 counts as appropriate);
7744 h. records of product testing, including microbial testing;
7745 i. any incidents that occurred during processing.

7746 **15.3.4. Records of storage and distribution of tissues and cells**

7747 Besides the information defined in Chapter 11 (on distribution and import/export), organisations
7748 undertaking storage of tissues or cells should keep at least the following records:
7749 a. storage location and a transfer record if storage locations change;
7750 b. date placed in storage;
7751 c. date removed from storage;
7752 d. records of storage temperature (where relevant);
7753 e. any incidents that occurred during storage.

7754 In addition, when the tissues or cells are transported or distributed to hospitals or clinics for
7755 application, tissue establishments should keep the following records:

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7756 a. name of party responsible for distribution;
7757 b. identification of the establishment, courier or individual who transported the tissues and cells at
7758 any stage between procurement and end use (clinical application);
7759 c. packaging records (e.g. records of the dry-shipper used);
7760 d. time and date of distribution of tissues and cells;
7761 e. time and date of delivery of tissues and cells;
7762 f. identification of the receiving establishment, clinician or ORHA;
7763 g. any incidents that occurred during distribution.

7764 **15.3.5. Records of clinical application of tissues and cells**

7765 Besides the information defined in Chapter 12, the ORHA should keep at least the following
7766 records:
7767 a. identification of the supplier tissue establishment;
7768 b. identification of the clinician or ORHA;
7769 c. type(s) of tissues and cells;
7770 d. product identification;
7771 e. identification of the recipient;
7772 f. date of clinical application;
7773 g. any incidents that occurred during clinical application;
7774 h. any adverse reactions or adverse events in the recipient;
7775 i. health outcomes of children born following MAR.

7776 Systems must be in place to assure the follow-up of tissue and/or cell recipients and children
7777 conceived after assisted reproductive technology (ART) treatment. Such follow-up can be achieved only
7778 if a close working relationship exists between all stakeholders: that is, the tissue establishment, ORHA,
7779 MAR/ART centre and parent(s) involved.
7780 Some national standards require the ORHA to provide the supplying tissue establishment with
7781 details of the patient to whom the tissues or cells were clinically applied. Whether this information is
7782 sent to the tissue establishment or not, it is essential that the end user maintain these records because
7783 ultimately they are responsible for recording the fate of the tissues or cells.

7784 ## 15.4. References

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7797 # Chapter 16. Biovigilance

7798 ## 16.1. Introduction

7799 This chapter provides general guidance on the implementation of good vigilance and surveillance (V&S)
7800 practice by all those (including regulators and Health Authorities) involved in the processes of
7801 transplantation, which includes medically assisted reproduction (MAR), from donation through banking
7802 to clinical use until the donated tissue or cell functions in the recipient. The tissue- and cell-specific
7803 chapters in Part B provide additional specific guidance on vigilance in those fields; in particular, Chapter
7804 27 details several specificities within MAR/ART (assisted reproductive technology) vigilance.
7805 A programme of V&S is essential for ensuring the quality and safety of tissues and cells for human
7806 application. The quality system focuses on preventing errors and maintaining a consistent standard of
7807 agreed specification for tissues and cells released for clinical application. However, occasionally,
7808 residual risks or procedural errors result in failures, disease transmissions or situations in which donors
7809 or patients are exposed to risk, even if not harmed. Reporting of these incidents presents important
7810 learning opportunities that can help all procurement organisations, tissue establishments (TEs), cell
7811 therapy and MAR facilities, and clinical users (not only those involved in the incident in question) to
7812 improve their processes and to achieve higher levels of safety and quality at all levels: from TEs to
7813 donors and recipients [1, 2].
7814 There are several stages (phases) in a biovigilance system. The first one is to detect and identify
7815 a biovigilance case that could be described as an adverse event or reaction. Depending on the case and
7816 the system in place at national level, the following steps can be done in parallel. After identifying the
7817 case, it must be reported or notified to the Health Authority even if the investigation is not concluded. If
7818 there is a suspicion that other centres could be affected or involved, they have to be alerted by the TE or
7819 by the Health Authority to prevent further complications. Every single case (at least every serious case)
7820 must be investigated and evaluated by the TE and Health Authority, with the collaboration of all centres
7821 involved and also a group of professionals with experience in infectious and malignant diseases, quality
7822 control and quality management, as well as professionals with experience in the use of the tissue
7823 involved in the case.
7824 Once the investigation is finished, it is important to decide how its findings should be managed,
7825 depending upon what kinds of actions have been decided on. Finally, the case will be closed and the
7826 final report must include both corrective and preventive measures. This final report should detail how
7827 to act on similar occasions in the future. It is worth noting that learning is an important benefit derived
7828 from biovigilance.

7829 ## 16.2. Definitions

7830 These adverse occurrences can be classified into 'adverse events' (AEs), which are process failures that
7831 might lead to harm in a recipient or living donor or to a loss of any irreplaceable autologous tissues or
7832 cells or to a loss of any highly matched allogeneic tissues or cells, and 'adverse reactions' (ARs), which
7833 are adverse outcomes that have indeed occurred with harm to a donor, a recipient or a child born through
7834 MAR procedures related to *in vitro* fertilisation (IVF) with gamete or embryo donation. An adverse
7835 event may or may not cause an adverse reaction. Similarly, an adverse reaction may or may not be related
7836 to an adverse event.
7837 According to European Union (EU) definitions, a 'serious adverse event' (SAE) in the present
7838 context is any untoward occurrence associated with the procurement (including donor selection), testing,
7839 processing, storage and distribution of tissues and cells that might lead to the transmission of a

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communicable disease, to a life-threatening, disabling or incapacitating condition for the patient or that might result in prolonged hospitalisation, morbidity or death. According to EU definitions, a 'serious adverse reaction' (SAR) is an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling or incapacitating, or which results in, or prolongs hospitalisation or results in morbidity.

These definitions are reflected in the World Health Organization (WHO) Notify Library for V&S of medical products of human origin (MPHO). Adverse outcomes are categorised in the library as follows:

a. Adverse reaction
   i. harm to a donor
   ii. harm to a recipient
   iii. harm to a fetus or offspring
b. Adverse event
   iv. risk of harm

In summary, an adverse reaction is an incident whereby a living donor, a recipient or a fetus or child created by IVF and intra-uterine insemination with donor gametes has been harmed, whereas an adverse event is an incident that results in a risk of harm, although no harm may actually occur. Those that are classified as 'serious' should be notified to Health Authorities, in accordance with national or regional (e.g. EU) requirements.

Although adverse incidents may occur at all stages from procurement to distribution of tissues and cells, many of them are not severe and may be managed through the quality management system (QMS) of the TE. Conversely, serious adverse reactions and events (SAREs) are rare. Therefore, there are significant benefits associated with consolidating V&S data on regional, national or international scales and on an integrated system for the different substances of human origin (SoHO), because they share exposure to risks from donation to transplantation (from breaches of ethical, legal and safety standards).

The follow-up of living donors after donation should ensure that, if a condition not known at the time of donation occurs to the donor, and it may have an impact on the recipient, it is clearly identified. In such cases there should be a documented procedure to notify the recipient's physician of this condition. This is not necessarily an adverse event. Conversely, when the recipient's physician detects an impact on the recipient, this must be reported to the TE. The same also applies to the potential long-term influence of any treatment provided for the procurement (e.g. mobilisation with cytokines or hormonal stimulation), in which case pharmacovigilance should also be involved.

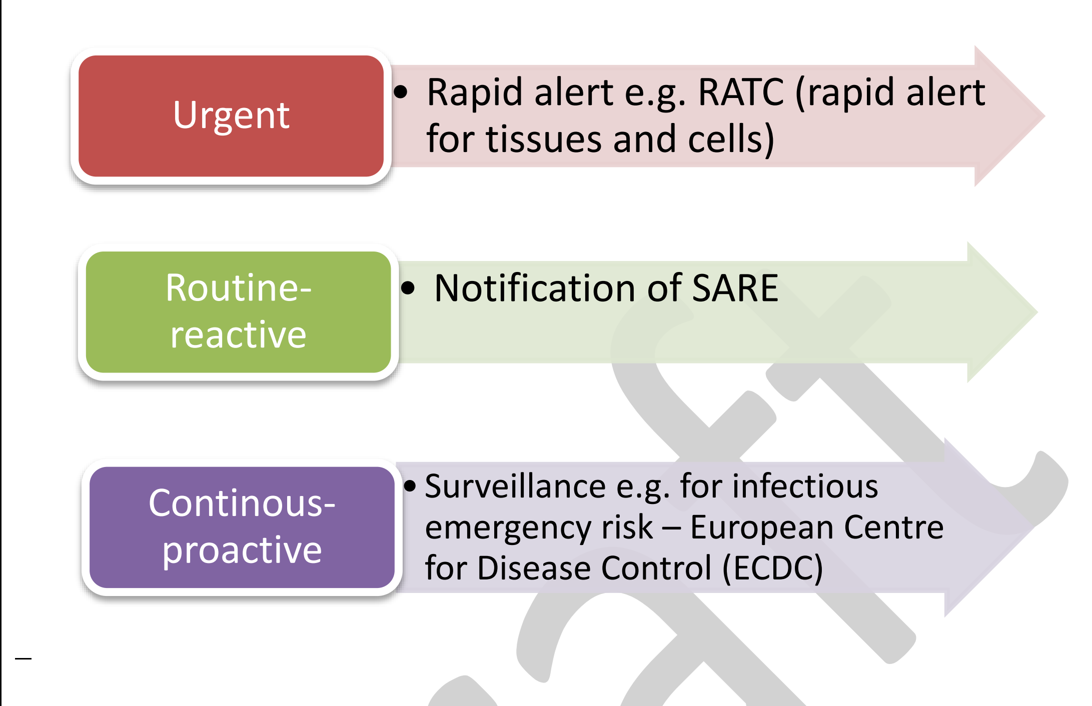
If products containing tissues or cells are classified as advanced therapy medicinal products (ATMPs) in the EU, the regulatory framework of pharmacovigilance must be applied. The relevant legal texts and guidelines are described on the pharmacovigilance web page of the European Commission [3]. Donation, procurement and testing of tissues and cells used to prepare an ATMP are regulated in the EU by directives on tissues and cells. Consequently, good communication between biovigilance and pharmacovigilance systems is essential to facilitate effective investigation and corrective/preventive actions if ATMPs are associated with adverse outcomes.

16.3. Management and quality of vigilance

16.3.1. Vigilance

As for other vigilance systems, vigilance activities in the field of tissues and cells should be considered and recognised at all levels of TEs that are authorised for tissue and cell activities. The organisation of the vigilance system, as well as the role of the various parties involved, should be defined and broadly communicated within the TE.

7887 Three levels of biovigilance can be described, depending on the types of measures and actions
7888 that can be taken into consideration: urgent communication, routine notification and proactive
7889 monitoring of possible SARs/SAEs (see Figure 16.1). These levels will be developed further in this
7890 chapter.
7891



7892 **Figure 16.1. Levels of biovigilance**

7893
7894 Health Authorities are encouraged to draw up guidelines for vigilance systems, notification forms,
7895 surveillance methods, acceptable risk criteria and examples of SAREs for tissues and cells that should
7896 be reported to them. Appropriate communication and co-ordination between procurement organisations,
7897 TEs and centres carrying out clinical application are essential for an efficient vigilance system.
7898 Organisations or bodies involved in activities based on tissues and cells (including clinical users) should
7899 have standard operating procedures (SOPs) in place that describe how to collect, report, investigate and
7900 communicate notifications for adverse reactions and events (AREs). Identification of a local co-
7901 ordinator, who has responsibility for V&S specified in their job description, is an effective measure. It
7902 is recommended that the QMS and V&S systems, both of which contribute to risk-management policy,
7903 should be co-ordinated at TE level according to guidelines established by the Health Authority and under
7904 the direct responsibility of the Responsible Person (RP). Implementation of computerised and integrated
7905 systems for collection and management of ARE data is encouraged.
7906 This chapter focuses on the procedures for detection, reporting, alert, investigation, management,
7907 evaluation and closure of AREs that may occur from donation until clinical application and follow-up.
7908 All AREs and non-compliances involving any party (clinical users, donors, patients or third parties) and
7909 including those with minor consequences, should be documented and reviewed regularly within the
7910 QMS of the TE. Each report or communication should be considered for classification as an SARE and
7911 should be managed as such if it meets the criteria described in this chapter. This allows trends to be
7912 monitored and actions to be taken to continually improve quality and safety.

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7913 **16.3.2. Surveillance**
7914 The term 'surveillance' denotes the follow-up of organs, cells or tissue recipients or living donors,
7915 with or without SARE, to provide indicators and information on stratification of risks. Furthermore, an
7916 active surveillance system should also monitor some specific expected serious side reactions or events.
7917 When a surveillance system is implemented, periodic analyses can show if there is an upward trend of
7918 SAREs, AEs or ARs, more or less systematically occurring and expected. These should be reported to
7919 the Health Authority, a root cause analysis should be initiated, and corrective measures should be
7920 implemented.
7921 Routine monitoring of clinical outcomes is part of the surveillance system. Clinical teams have to
7922 set up registries with follow-up on grafts and recipients post-transplant, as well as living donor
7923 outcomes, in order to monitor the results and to identify currently unknown risk factors. This monitoring
7924 should be complemented by an active surveillance system for well-known adverse reactions.
7925 Unfortunately, in the case of tissues there are very few registries, and most of them are at national level,
7926 e.g. cornea registry (see Chapter 17). Although they are outside the scope of the vigilance system –
7927 because this focuses only on the "undesired and unexpected SAR" – they should be evaluated further in
7928 order to exclude their occurrence being caused by a systematic error (e.g. incorrect handling of heart-
7929 for-heart valves during procurement).
7930 The expected frequency of these 'known SARs' can be defined by the relevant experts or
7931 professional bodies, based on local experience and evidence based on literature data (e.g. expected rate
7932 of arterial thrombosis after the use of cryopreserved arteries). When the frequency of these complications
7933 increases in one centre beyond a threshold of pre-defined rates, a notification of "suspected SAR" must
7934 be made to the Health Authority. The root cause analysis has to be performed by the local investigation
7935 team in order to determine the reasons for this deviation from the acceptable rate. This investigation
7936 falls within the framework of the vigilance system, in order to trigger corrective measures and to improve
7937 quality of care to patients. But such surveillance should also identify whether good results are achieved
7938 by risk-avoiding behaviour of an institution or by true best clinical practice applied.
7939 The widespread use of active surveillance systems will be a step-by-step process that still requires
7940 healthcare professionals to obtain consensus views on some important points, including issues like the
7941 definition of serious adverse reactions and events, as well as the description of their appropriate
7942 monitoring.

7943 16.4. Adverse reactions

7944 Adverse reactions must be detected, reported, investigated and assessed in terms of severity,
7945 imputability, probability of recurrence or frequency, and consequences. Efficient systems for rapid
7946 quarantine or recall of unsafe tissues or cells must be in place, along with procedures for look-back
7947 where donors or recipients are found to have been exposed to a risk. Important learning outcomes from
7948 each adverse reaction should be communicated appropriately to all professionals involved.
7949 Several symptoms or situations can suggest that an adverse reaction might have occurred in a
7950 recipient of a tissue or cells and should, therefore, be seen as 'triggers' for an adverse reaction report.
7951 Note that, in certain circumstances, clinicians may knowingly transplant an infective donation (e.g.
7952 *Cytomegalovirus*-positive bone marrow); in such cases, patients should be informed about the benefits
7953 and the additional risks, and there should be specific follow-up. Clinical and biological monitoring, as
7954 well as prophylactic or pre-emptive treatment, should comply with existing recommendations or
7955 regulatory requirements, where they exist. Below are examples of reportable adverse reactions (for more
7956 information, see the chapters in Part B on each specific tissue) [with abbreviated descriptions in square
7957 brackets]:
7958 a. suspected harm in living donor related to procurement [Donor harm];
7959 b. unexpected primary infections possibly transferred from donor to recipient (e.g. viral, bacterial,
7960 parasitic, fungal, prion) [Infection from donor];

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c. suspected transmitted infection (viral, bacterial, parasitic, fungal, prion) possibly due to contamination or cross-contamination by an infectious agent in the procured tissues, cells or associated materials, between procurement and their clinical application [Infection from infected/contaminated tissues and cells];
d. hypersensitivity reactions, including allergy, anaphylactic reactions or anaphylaxis [Hypersensitivity];
e. malignant disease possibly transferred by the tissues or cells (donor-derived, process-associated or other) [Malignancy];
f. unexpectedly delayed or absent engraftment, or graft failure (including mechanical failure) [Failure];
g. toxic effects to tissues and cells or associated materials [Toxicity];
h. unexpected immunological reactions due to tissue or cell mismatch or, in the case of ART, mismatch between oocytes and sperm in a partner donation [Mismatch];
i. aborted procedure involving unnecessary exposure to risk, e.g. wrong tissue supplied, discovered after patient is anaesthetised and the surgical procedure has begun [Undue risk];
j. suspected transmission of genetic disease by transplantation or gamete/embryo donation [Genetic abnormality];
k. suspected transmission of other (non-infectious) illness [Other transmission];
l. transfusion-associated circulatory overload in haematopoietic progenitor cell transplantation [Volume overload];
m. neurological reaction [Insult];
n. severe febrile reaction [Fever];
o. other [Other].

16.4.1. Detection of adverse reactions

Effective V&S relies heavily on all health professionals involved, from procurement to clinical application, namely:

a. medical staff (including surgeons) involved in tissue- and cell-procurement activities who might become aware or informed of additional safety information on donors during their follow-up;
b. staff and personnel carrying out procurement of tissues and cells;
c. clinical users who should pay attention to adverse outcomes and be aware when such outcomes might be associated with the clinical use of tissues or cells;
d. physicians caring for children born after non-partner MAR/ART treatment who may detect a genetic abnormality and, by reporting it, prevent further distribution of gametes/embryos from that donor;
e. any other TE staff involved in any procurement and transplant activities;
f. other vigilance systems (e.g. haemovigilance, material/device vigilance, pharmacovigilance) when issues of concern are detected that might affect the safety of tissues or cells for transplantation.

Adverse outcomes might result from many diverse factors associated with the surgical procedure or the patient's underlying condition. Hence, clinicians might not consider the tissues or cells that were applied to be a possible source of the adverse outcome. TEs that supply tissues and cells should encourage procurement organisations and clinical users of tissues and cells to always consider whether adverse outcomes might have been associated with the donation process or caused by the tissues or cells applied, so that similar occurrences are prevented in the future.

For most types of well-established clinical application of tissues and cells, detailed reporting of clinical outcome by the clinical user to the TE is required only in those exceptional circumstances in which there is suspicion of an untoward adverse reaction. However, reporting of the clinical progress of tissue and cell recipients to the TE might also be required for all highly matched, life-saving transplants

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such as HPC infusions, or when novel tissue or cell processes have been applied or new types of tissues or cells are being transplanted. This routine clinical follow-up is not considered as part of vigilance.

An important part of vigilance is detecting donation complications (also considered to be adverse reactions) in living donors that might be associated with the donation process in some way. For example, adverse reactions may be detected after stimulation treatment in living donors and recipients (see Chapters 22 on HPC and 27 on MAR).

16.4.1.1. Surveillance for new risks

Surveillance programmes should include an activity of scanning for new risks that have not been recognised previously. New risks may be related to new donors, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing. Newly emerging infectious diseases, for which targeted testing can be carried out or which might imply the need to exclude certain donors, represent an example of one type of new risk. The European Centre for Disease Prevention and Control (ECDC) monitors the epidemiology of diseases in Europe and publishes a weekly *Eurosurveillance* report that provides useful data to support the development of donor-selection policy. Moreover, the ECDC has recently been mandated to initiate risk assessment on particular epidemic agents, infectious diseases or new *in vitro* diagnostic techniques in the field of tissues and cells.

16.4.2. Reporting adverse reactions

16.4.2.1. Clinicians to tissue establishments

TEs that supply tissues and cells should provide organisations representing clinical users with clear instructions on how to report adverse reactions, preferably using standardised documentation. In general, suspected adverse reactions should be reported immediately by the clinical users to the TE that supplied the tissues or cells before investigation or confirmation. This approach allows the TE to take appropriate precautionary actions to prevent harm to other patients, and start the investigation process. Clinical users should be encouraged to report all types of suspected adverse reactions (serious and non-serious) which might be related to the tissues and cells from the supplying TE, to allow filtering of those considered to be serious and reportable to an authority at a later stage.

Specifically, in MAR, couples undergoing non-partner donations are important stakeholders when reporting of SARs is concerned. Clinicians treating patients with donor gametes should inform and encourage patients that, if any disease is detected in their donor child, they must report back to the MAR/ART centre. Patients should be clearly informed of their registration obligations concerning diseases in donor children. It is in the interest of all patients using donor gametes that SARs in donor children are quickly notified in order to be able to quarantine straws from these specific donors and prevent further spread of a particular disease. It is imperative to note that not all diseases in donor children are directly related to the donor. Therefore, a careful risk assessment is needed where the type of disease (chromosomal, multifactorial, single-gene or mitochondrial disorders, communicable and noncommunicable diseases) and the possibility of (genetic) testing of the patient and/or donor (if possible), as well as global prevalence and genetic predisposition, should be taken into account.

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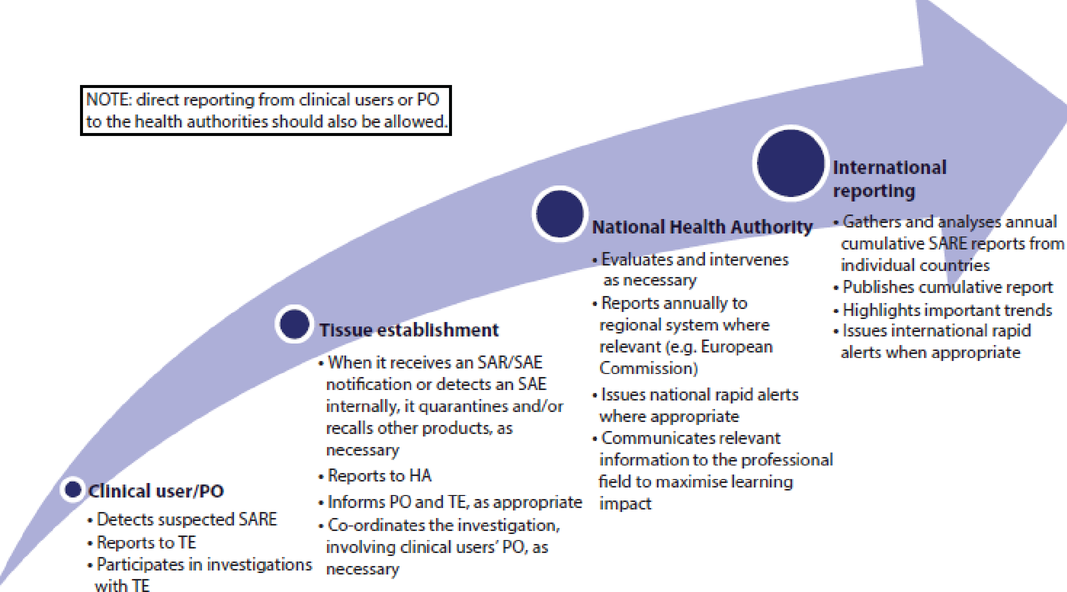

8049 **Figure 16.2. Reporting flow for serious adverse events/reactions**

8050

8051 *16.4.2.2. Procurement organisations to tissue establishments*

8052 Similarly, health professionals and procurement organisations should report adverse reactions in
8053 living donors and recipients to the TE, even if the adverse reaction is only suspected to be donation-
8054 derived, so that the broader implications for other centres and donors can be considered without delay.

8055 *16.4.2.3. Reporting to regional/national programmes*

8056 TEs must report information on SARs to Health Authorities (see Figure 16.2). In the EU, all SARs
8057 related to quality or safety that meet the descriptions of 'serious', 'life-threatening' or 'death' must be
8058 reported to the Health Authorities.

8059 The TE is responsible for providing clinical-user entities, procurement organisations and critical
8060 third parties with clear instructions, forms and guidance on how to notify adverse reactions in accordance
8061 with national or local requirements. Reporting and management of adverse reactions should be
8062 incorporated in the quality system of the TE, with one or more SOPs that describe the process for
8063 acknowledgment of notifications, investigation, follow-up on corrective and preventive actions and
8064 reporting to the Health Authorities if criteria are met. Moreover, a specific procedure should enable rapid
8065 action, if needed, to be taken by all affected organisations to protect the safety of recipients. This may
8066 involve tissue and cell quarantine, recall and look-back in patients who have already had implicated
8067 tissues or cells applied. These actions may need to be taken by organisations other than the one that
8068 received the original notification. For example, the organ procurement organisation will play a central
8069 part when the donor was an organ and tissue donor.

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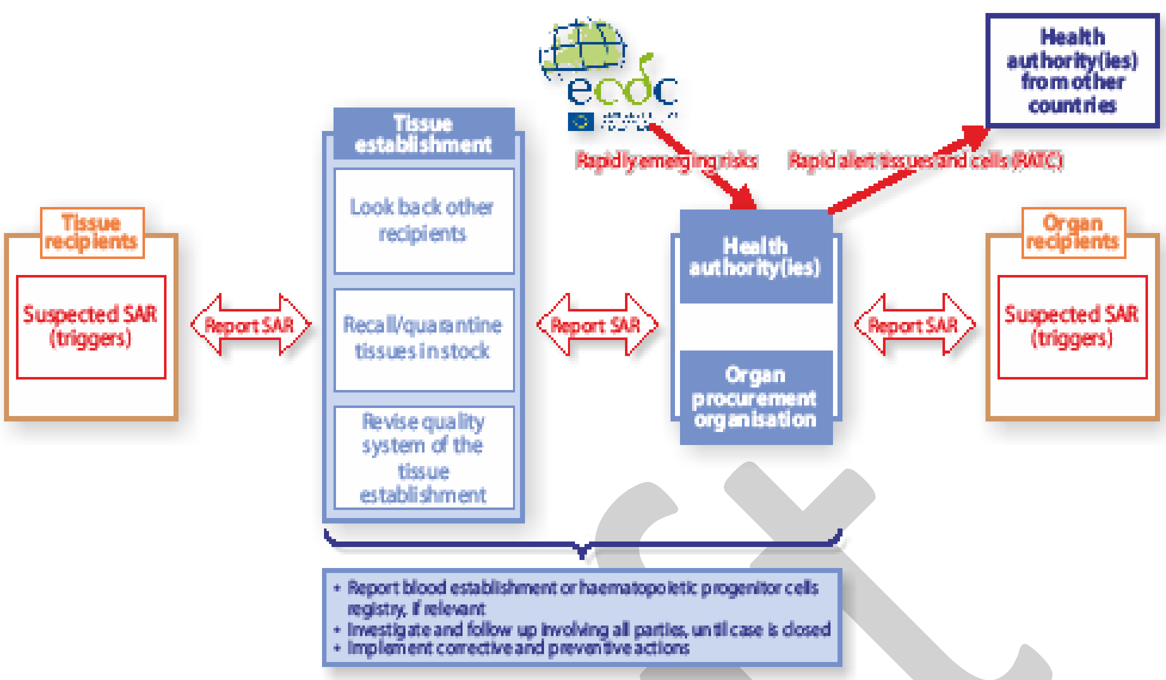

Figure 16.3. Example of an adverse reaction involving multiple parties

Figure 16.3 shows a series of actions that might need to be taken in a report of suspected transmission from a deceased donor of organs and tissues. It makes clear that communication with other organisations that might need to quarantine implicated tissues or cells, or conduct recalls or look-backs, should be quick and effective.

Although reporting of SARs should, in general, be co-ordinated and centrally reported by TEs at a national level, it is recommended that national V&S programmes allow direct reporting from clinical users or even patients to Health Authorities. This might occur where a clinician or a patient suspects that a TE is not working correctly or where they do not have confidence, for whatever reason, that the report will be fully investigated.

16.4.2.4. International reporting

If SARs are detected in relation to tissues or cells that have entered international distribution channels, appropriate international collaboration should ensure that all the stakeholders involved (clinicians, TEs and Health Authorities) in each of the countries concerned are informed and participate, as necessary, in the investigation and follow-up actions.

EU member states are obliged to send an annual report of the SARE notifications that they have received to the European Commission [4]. Such international reporting allows for trend analyses on the basis of consolidated data and for sharing of the lessons learned.

At national level, the Health Authority or regulatory agency mandates SARE reporting for donors (as described above for the EU). However, HPC registries have implemented a global reporting system because HPC products have specific characteristics (see Chapter 22).

16.4.2.5. Follow-up of the donors of tissue and cells

The follow-up of donors of tissue and cells is important for the early detection and reporting of SAREs. Short-term follow-up is essential to ensure recovery from the donation procedure. Long-term follow-up is desirable to enable any long-term effects of the donation to be identified. The nature and duration of this follow-up depend on the type of donation, the nature of the intervention and its potential impact on the individual's health [5].

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Completed donor follow-up has been defined as physical, phone or laboratory contact at a given time point [6]. There should be written SOPs for follow-up of donors. For related donors, the responsible person for the follow-up should be the physician undertaking the assessment of the donor for the donation. For unrelated donors, the follow-up should be the responsibility of the relevant donor registry, if present.

After donation, the living donor should also be followed up, by documented procedures. This follow-up will depend on the type of donation: for example, no donor follow-up is required for cord blood donation, whereas HPC donors will require a minimum of 5 years. The length of follow-up should reflect the guidance of the professional body. This follow-up ensures that, if a new condition occurs that may have an impact on the recipient, proper action can be taken. This should clearly be documented. No matter how extensive the testing that is performed prior to donation, the donor can develop diseases not known at the time of donation. These can be newly discovered infectious diseases or malignancies. These conditions may have been transmitted to the recipient as well. There is no clear evidence regarding malignancies, but haematological malignancies within one year of donation may have been present in the graft and transmitted to the recipient. In these cases, the recipient should be specifically monitored for the presence of the disease and if possible, preventive steps should be taken.

16.4.3. Investigation and assessment of adverse reactions

Depending on the level at which the adverse reactions occurred, certain measures have to be taken before starting the investigation. The first precautionary measure is to quarantine any other tissues or cells from the same donor if there are any in storage. If tissues or cells have already been distributed, it must be determined where they are allocated and consider the possibility of recalling. These measures are intended to minimise the number of recipients exposed to the same reaction.

a. The first step in the investigation is to determine the severity. A 'severity scale' can be used to decide whether a particular adverse reaction is an SAR that needs to be reported to the Health Authorities. The scale shown in Table 16.1 is used in the EU. It was proposed by the project European Union Standards and Training for the Inspection of Tissue Establishments (EUSTITE) [7] for vigilance for tissues and cells and is based on the scale used for haemovigilance.

Table 16.1. Severity scale for serious adverse reactions

Not reportable	Insignificant	No harm to the recipient or living donor, and considered to be reportable as an event rather than a reaction according to EU directives
	Non-serious	Mild clinical consequences that do not necessitate hospitalisation and/or do not result in long-term disability or consequences for the recipient or living donor
To be reported	Serious	Adverse reaction resulted in: • hospitalisation or prolongation of hospitalisation and/or • persistent or significant disability or incapacity and/or • medical or surgical intervention to preclude permanent damage or impairment of a body function and/or • evidence of a serious transmissible infection and/or • birth of a child with a serious genetic disease after MAR with non-partner gametes or donated embryos
	Life-threatening	The living donor or recipient required major intervention after procurement or application of tissues or cells (vasopressors, intubation, transfer to the intensive care unit) to prevent death and/or there is evidence of a life-threatening transmissible infection
	Fatal	Death in a living donor or a recipient of tissues or cells

Adverse reactions in recipients of tissues or cells should be investigated by a team that can carry out an independent investigation; it should include the clinician who transplanted the tissues or cells, the TE that provided them and, in more serious cases, the Health Authority in that country. Efficient co-ordination of the investigation is critical to rapid implementation of

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8134 effective corrective actions. If relevant, experts in particular fields (e.g. viral transmission)
8135 should also be invited to participate in the investigation of the adverse reaction.
8136 b. The second step is to assess imputability. The investigation should focus on establishing the
8137 level of imputability (i.e. the extent to which the tissues or cells used clinically can be
8138 considered to have caused the adverse reaction). The scale provided in Table 16.2, developed
8139 by EUSTITE, can be applied to describe the outcome of an imputability investigation. It
8140 proposes that all adverse reactions be graded in terms of imputability. Table 16.2 also
8141 recommends specific approaches to the establishment of imputability for suspected infectious
8142 or malignant transmissions, as proposed by Garzoni and Ison in the context of transplantation
8143 [8]. Imputability grades might change during an investigation and should, in general, be
8144 assigned at the point of initial notification and again at the completion of the adverse reaction
8145 investigation. The evaluation of imputability should be based on scientific or clinical data.
8146 The ECDC, the WHO or other sources of epidemiological and risk information may be useful
8147 to support the process.
8148

8149 **Table 16.2. Scale describing possible outcomes of an imputability investigation**

	Criteria adapted from EUSTITE-SoHO V&S [9]	*Criteria for infectious and malignant transmissions, adapted from the Disease Transmission Advisory Committee [10, 11]*
Not assessable	Insufficient data for imputability assessment	Insufficient data for imputability assessment
0. Excluded	Conclusive evidence beyond reasonable doubt for attributing an adverse reaction to alternative causes	Suspected transmission and fulfilment of at least one of the following conditions: • clear evidence of an alternative cause • the appropriate diagnostic tests carried out have failed to document infection by the same pathogen in any recipient from the same donor • laboratory evidence that the recipient was infected with the same pathogen or had a tumour before the application of organs, tissues or cells
1. Possible	The evidence is indeterminate for attributing an adverse reaction to the quality/safety of tissues and cells, to the donation process or to alternative causes	Either • suspected transmission and • laboratory evidence of the pathogen or tumour in a single recipient or • data suggest a transmission but are not sufficient to confirm it
2. Probable	The evidence is clearly in favour of attributing the adverse reaction to the quality/safety of tissues and cells (for recipients) or to the donation process (for donors)	The following two conditions are met: • suspected transmission and • laboratory evidence of the pathogen or tumour in a recipient And at least one of the following conditions is met: • laboratory evidence of the same pathogen or tumour in other recipients • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, such evidence must indicate if the same recipient was negative for the pathogen involved before transplantation
3. Definite; certain	The evidence is conclusive beyond reasonable doubt for attributing the adverse reaction to the quality/safety of tissues and cells (for recipients) or to the donation process (for donors)	All the following conditions are met: • suspected transmission • laboratory evidence of the pathogen or the tumour in a recipient • laboratory evidence of the same pathogen or tumour in other recipients (if multiple recipients) • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, it should be noted whether the same recipient was negative for the pathogen before transplantation

8150
8151 Consideration should also be given to the practice of keeping pre-transplant serum archives for
8152 transplant recipients to support imputability investigations.
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8154 TEs have to review:
8155 • 1. all the reagents, substances etc. that were in contact with the tissues and cells during
8156 processing, checking the expiry date and the sterility;
8157 • 2. the tissues processed in the same room on the same day, the day before and the day after;
8158 • 3. all the microbiological results of the donor and of each specific graft;
8159 • 4. the microbiological checks of the processing rooms.

8160 The ORHA that uses the tissues or cells needs to determine if other patients were operated on in
8161 the same operating room and their clinical and microbiological conditions. This approach is not advised
8162 for recipients of gametes or embryos (see Chapter 25).

8163 16.5. Adverse events

8164 Adverse events can occur at any moment from donor selection to clinical application. However it should
8165 be emphasised that an adverse event may not always produce a subsequent reaction in the recipient.
8166 Non-compliances with the quality system should be documented and investigated as part of the
8167 internal QMS. On occasions, however, a particular non-compliance may be of such importance that it
8168 should be considered an SAE and reported through the vigilance system. Some examples of SAEs are
8169 (for more information, check the specific tissue chapter in Part B):
8170 a. Final result of a negative-to-date release with a cultured cornea was reported as positive; no
8171 adverse reaction detected in the cornea recipient.
8172 b. Aspergillus was detected in an incubator used for storing tissues and cells that have since been
8173 distributed.
8174 c. Loss of an embryo during manipulation of the culture dish; the patient requires new cycle of
8175 IVF.
8176 d. Skin donor not tested for malaria although his residence was in a malaria-endemic area.
8177 e. Lack of liquid nitrogen in a liquid nitrogen refrigerator containing several tissues, resulting in
8178 thawing of the tissues.
8179 f. When a frozen bone arrives at the hospital and the bag is broken.
8180

8181 16.5.1. Detection of serious adverse events

8182 For effective detection of adverse events, all relevant stakeholders must be aware of their
8183 responsibilities for identifying errors or unexpected results. This includes all staff in TEs and
8184 procurement organisations, those working in organisations such as testing laboratories that provide 'third
8185 party' services to TEs, and clinical users who may also detect errors at the point of clinical use. In EU
8186 Directive 2006/86/EC, the definition of an SAE includes those incidents often referred to as 'near
8187 misses', i.e. where an error or fault is detected and corrected without causing harm.

8188 16.5.2. Serious adverse event reporting

8189 According to instructions from the European Commission to EU member states for annual
8190 vigilance reporting, deviations from SOPs in TEs (or other adverse events) that have implications for
8191 the quality and safety of tissues and cells should result in an SAE report to the Health Authority if one
8192 or more of the following criteria [12] apply (see also Figure 16.1):
8193 a. inappropriate tissues or cells have been distributed for clinical use, even if not used;
8194 b. the event could have implications for other patients or donors because of shared practices,
8195 services, supplies or donors;
8196 c. the event resulted in a mix-up of gametes or embryos;
8197 d. the event resulted in a loss of traceability of tissues or cells;

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e. the event resulted in loss of any irreplaceable autologous tissues or cells or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;

f. the event resulted in loss of a significant quantity of unmatched allogeneic tissues or cells.

16.5.3. Investigation and assessment of serious adverse events

Despite the fact that SAEs, by definition, have not (or not yet) involved harm to recipients or donors, the impact of an SAE can be significant if considered in a broader way. The impact assessment tool given in Appendix 24 can also be applied to SAEs to help reach a decision on the response required.

16.6. Vigilance co-ordination

Co-ordination between various systems of vigilance (e.g. organ and blood vigilance, medical device vigilance, pharmacovigilance) should be in place at the local level (TE) and at the Health Authority level.

The lack or omission of the exchange of information can put more patients or the same recipients at risk. Sometimes it is someone within the system who discovers a problem and needs to inform the Health Authority. For example, if there is problem with a bag used for cryopreserved tissues or cells and that problem altered the characteristics of the product, or if such a bag was stored at −196 °C and after some time this bag was broken, then the medical device vigilance system should be informed. The same caution should apply when faced with an SAR or SAE with organs if it involves tissues which were retrieved, or vice versa; in either case, all the corresponding vigilance systems should be informed.

16.6.1. Rapid alerts

In some circumstances, a particular event or reaction requires rapid communication nationally or internationally to facilitate urgent action, such as a recall of products or critical materials or the quarantine of tissues or cells. In that case a communication system must be available at all times. Rapid alerts should only be issued in exceptional circumstances. The following criteria have been identified in the SoHO V&S project [13] as triggers for rapid alerts within or between EU member states:

a. an ARE of a serious or potentially serious nature;

b. potential risk to other individuals or other TEs;

c. wider public health implications;

d. rapid intervention needed (preventive or corrective measures, urgent communication).

Within the EU, a system for rapid alerts – called Rapid Alerts for Tissues and Cells (RATC) – is hosted by the European Commission and enables the competent authorities of EU member states to rapidly share urgent information on risks to patients where that information has consequences in more than one EU member state. In February 2013, this system was moved to a new secure internet platform where all rapid alerts are generated and shared, with access restricted to Competent Authorities.

16.7. Vigilance communication: education and training

16.7.1. 'No blame' culture

Effective communication of the results of vigilance systems is fundamental to ensure that the benefits of these programmes are realised in practice. Regular feedback to health professionals is critical to support continued notification of AREs. All stakeholders, Health Authorities, TEs and clinicians should promote a culture that encourages reporting in a non-punitive context for the benefit of patients and donors. It should be accepted that mistakes happen and that no programme of transplantation or MAR is risk-free. Programmes of training and awareness should be organised to encourage reporting. The message should be promoted that reporting and disseminating V&S information can result in positive improvements for donors and patients, as well as feedback to health professionals.

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8241 ### 16.7.2. Vigilance experience and feedback

8242 Health Authorities and professional societies should publish the results of their programmes
8243 without identifying individual centres, hospitals or individual people. Those TEs or hospitals directly
8244 involved in specific incidents should also consider publishing their experience to alert others to the
8245 means by which they detected and confirmed the event or reaction.
8246 The Notify Library is an initiative launched by the WHO and supported by the Italian National
8247 Transplant Centre (CNT) that has gathered information on documented adverse occurrences in
8248 transplantation and assisted reproduction. It has reviewed cases to identify general principles supporting
8249 detection and investigation. The database has been constructed from the information gathered and is
8250 accessible on a dedicated website [14, 15]. The database is maintained and updated on this platform and
8251 is intended as a communication hub for institutions and organisations worldwide collaborating in the
8252 facilitation of access to V&S information to improve safety and efficacy.

8253 ### 16.7.3. Educational training and workshops

8254 When SoHO are used, there is always a risk that needs to be considered if something happens
8255 afterwards that can be related to the quality of the tissue or cells used and needs to be communicated.
8256 Health Authorities should encourage biovigilance awareness in all professionals involved in tissues and
8257 cells at any step (from donation to implant). To achieve this awareness, it is necessary to educate and
8258 train the professionals about the benefits of implementing a biovigilance system. This can be done with
8259 a high-quality educational programme and with well-organised workshops, disseminating the message
8260 in meetings, publishing reports with anonymised data, etc.
8261 The purpose is to stimulate reporting in an appropriate manner, but avoiding over-reporting, which
8262 can collapse the system. Professionals need guidance about what to communicate, when, and to whom.
8263 Healthcare providers need to have confidence in this system, which is why it is important that the
8264 reporting system is non-punitive and confidential. Reporting and further analysis are very useful tools
8265 for learning how to avoid mistakes and other errors; in the end, the resulting analysis is beneficial for
8266 the safety of donor and patients.
8267 Workshops – using real cases for discussion, describing how to investigate them and defining the
8268 possible causes of SAREs summarised in the final reports – can help in professionals' daily work.

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Related documents:
Appendix 24. Serious adverse reaction or event: impact assessment tool

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8312 PART B – TISSUE SPECIFIC 8313 REQUIREMENTS

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Chapter 17. Ocular tissue

17.1. Introduction

Ocular tissues procured from deceased donors are used for treating loss of vision caused by corneal disease or trauma, as well as for reconstructive and glaucoma surgery. The cornea is the principal refractive component of the eye. Good vision depends on corneal transparency and a smooth, spherical ocular surface. The cornea is also part of the outer coat of the eye and must therefore be strong enough to withstand the intraocular pressure and help protect the delicate inner structures of the eye.

17.1.1. Corneal transplantation

A corneal transplant (keratoplasty) is an operation to remove all or part of a diseased or damaged cornea and replace it with healthy donor tissue. In Europe, the main indications for corneal transplantation include:

- Fuchs endothelial dystrophy (FED) – failure of the corneal endothelium, the monolayer of non-dividing cells lining the inner surface of the cornea that controls stromal hydration and thus maintains corneal transparency.
- Keratoconus – a connective tissue disorder resulting in thinning of the corneal stroma, which normally accounts for 90 % of corneal thickness, and distortion of corneal shape.
- Pseudophakic bullous keratopathy (PBK) – failure of the corneal endothelium as a consequence of previous cataract or other intraocular surgery.
- Infection, such as *Herpes simplex* keratitis (HSK) – mainly treatment of post-infectious scars, but acute infection may require a therapeutic keratoplasty.
- Regraft for a failed corneal transplant.

These conditions may all be treated with a full-thickness corneal transplant (penetrating kerato-plasty, PK); however, current practice is to replace, where possible, only the dysfunctional part of the cornea [1, 2, 3] as illustrated in Figure 17.1.

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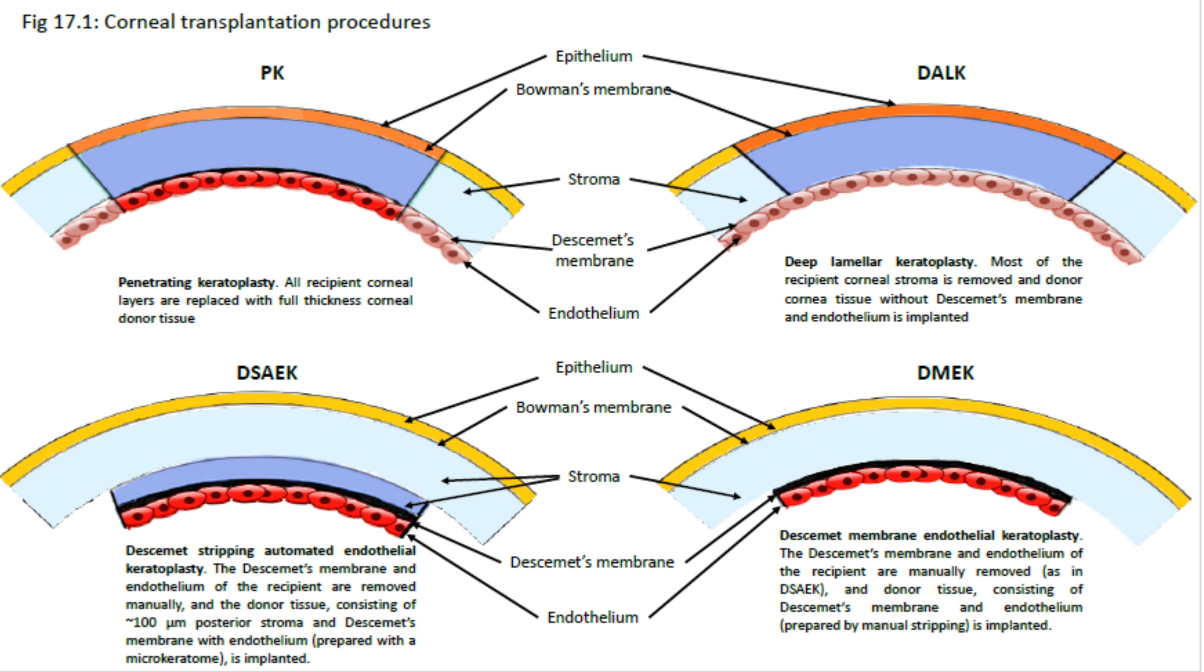
Figure 17.1 - Figure under elaboration



Endothelial keratoplasty (EK) is the method of choice for endothelial dysfunction such as FED or PBK. The graft lamella, which consists of endothelium on its basement membrane (Descemet membrane), with or without a thin supporting layer of stroma, is inserted through a small incision into the anterior chamber of the eye and attaches to the posterior surface of the patient's cornea. The initial attachment is ensured by air or sulphur hexafluoride gas tamponade of the anterior chamber. The affected patients are mostly elderly and the advantages of EK over PK include much faster visual rehabilitation, lower rejection rates and, since there are no sutures required to hold the graft in place, less surgically induced astigmatism. There are currently four techniques for preparing tissue for EK:

- Descemet stripping endothelial keratoplasty (DSEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared by manual dissection.
- Descemet stripping automated endothelial keratoplasty (DSAEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared using a microkeratome.
- Descemet membrane endothelial keratoplasty (DMEK) – endothelium on Descemet membrane without any stroma, prepared by manually separating Descemet membrane from the stroma.
- Pre-Descemet membrane endothelial keratoplasty (PDEK) – endothelium on Descemet membrane with a thin layer of pre-Descemet stroma, prepared by injecting air to create a 'big bubble', which separates the graft from the bulk of the stroma.

For keratoconus, which typically affects younger patients, deep anterior lamellar keratoplasty (DALK) is an alternative to PK that replaces the full thickness of the stroma, leaving the recipient's endothelium intact. As immunological rejection directed against the endothelium is one of the main reasons for PK failure, the advantage of DALK is that the patient's endothelium cannot be rejected. Superficial stromal defects and scars can also be treated by anterior lamellar keratoplasty (ALK) but without the need to replace the full thickness of the stroma.

Tissue for PK and EK requires a corneoscleral disc with a viable endothelium. Tissue for DALK and ALK does not require a viable endothelium; however, corneoscleral discs with an endothelium suitable for PK may be requested for DALK owing to the occasional need to switch procedure in the

8370 operating room from DALK to PK. Tissue for EK may be prepared in a tissue establishment, which
8371 saves time for the surgeon and avoids the risk of damage to the tissue when prepared in the operating
8372 room.
8373 Corneal transplant outcome – in terms of both graft survival and visual outcome – depends on the
8374 indication and reason for transplantation, the presence of pre-operative risk factors, such as vascularised
8375 cornea, glaucoma and inflammation, and post-operative complications such as allograft rejection. In the
8376 absence of other risk factors, keratoconus and FED are considered to be low-risk grafts with 5-year
8377 survivals of, respectively, 95 % and 82 % [4]. Regrafts and PBK are more likely both to suffer allograft
8378 rejection and to fail and are therefore considered higher-risk grafts with 5-year survival of, respectively,
8379 56 % and 54 % [4]. A major cause of graft failure is allograft rejection. Most rejection episodes can be
8380 successfully treated with topical (sometimes intraocular or systemic) corticosteroids. In some cases, use
8381 of systemic immuno-suppression (e.g. cyclosporine, tacrolimus, mycophenolate) is considered
8382 necessary to reduce the risk of immune reactions. The results from studies of human leukocyte antigen
8383 (HLA) matching to reduce the risk of rejection are not as conclusive as for organ transplantation, but
8384 matching may be beneficial for high-risk corneal grafts [5].

8385 **17.1.2. Further use of ocular tissue**
8386 If, for example, a patient has suffered multiple failed corneal grafts, a keratoprosthesis may be an
8387 appropriate alternative to a corneal graft [6]. Keratoprostheses are attached to a ring of corneal tissue
8388 from a donor, which is then sutured to the recipient's cornea after removal of the failed graft. Sclera and
8389 cornea can also be used in glaucoma surgery and for reconstructive surgery of the ocular surface. Limbal
8390 tissue, which contains a population of corneal epithelial progenitor cells, may be transplanted as a
8391 keratolimbal allograft (KLAL) to treat ocular surface disease caused by failure of the corneal epithelium
8392 (limbal stem cell deficiency) [7].
8393 There are other tissues and cells that are used in ocular surgery but not covered in this chapter:
8394 • Limbal progenitor cells may be isolated from a corneoscleral disc and expanded *ex vivo* for
8395 treating ocular surface disease (see Part C, Chapters 29 and 30) [8];
8396 • Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for
8397 injection as a suspension into the anterior chamber for treatment of corneal endothelial disease
8398 (see Part C, Chapters 29 and 30) [9];
8399 • Amnion is used for treating ocular surface conditions and as a support for limbal progenitor
8400 cells (see Chapter 18) [10];
8401 • Autologous and allogeneic serum eye drops may be used for treating dry eye (see Part C,
8402 Chapter 35) [11].

8403 The following generic chapters of this Guide (see Part A) all apply to ocular tissue banking and
8404 must be read in conjunction with this chapter:
8405 a. Introduction (Chapter 1),
8406 b. Quality management, risk management and validation (Chapter 2),
8407 c. Recruitment of potential donors, identification and consent (Chapter 3),
8408 d. Donor evaluation (Chapter 4),
8409 e. Donor testing (Chapter 5),
8410 f. Procurement (Chapter 6),
8411 g. Premises (Chapter 7),
8412 h. Processing (Chapter 8),
8413 i. Storage and release (Chapter 9),
8414 j. Principles of microbiological testing (Chapter 10),
8415 k. Distribution and import/export (Chapter 11),
8416 l. Organisations responsible for human application (Chapter 12),
8417 m. Computerised systems (Chapter 13),

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n. Coding, labelling and packaging (Chapter 14),
o. Traceability (Chapter 15),
p. Biovigilance (Chapter 16).

This chapter defines the specific requirements for ocular tissues that vary from the generic chapters in Part A of this Guide. Where differences are not specified, the requirements of the relevant generic chapter should be followed.

17.2. Donor evaluation

17.2.1. Tissue-specific exclusion criteria for ocular tissue donation

Acceptance and exclusion criteria for cornea donation that differ from the criteria for other tissues are based on the avascularity of the cornea and ocular-specific conditions that may affect the cornea.

17.2.1.1. Selection criteria for ocular tissue other than cornea

For any tissue or cells that are not derived from the avascular cornea, such as sclera, limbal tissue and limbal cells, the exclusion criteria for non-ocular tissues must be followed (see Chapter 4).

17.2.1.2. Donor age

Provided that corneas are examined to exclude those unsuitable for transplantation based on endothelial cell density and/or stromal abnormalities (see §17.7), the upper age limit for eye donors may be determined by the tissue establishment. The likelihood that corneas will be suitable for PK or EK does decline with increasing donor age but, where the endothelial cell density is considered sufficient, graft survival is little affected by donor age [12, 13]. The minimum donor age is more uncertain and should be determined by the tissue establishment because corneas from young children lack rigidity and have a high radius of curvature. There is likely, therefore, to be little demand for corneas from such young donors for transplantation; however, corneas from these donors may be important as a source of limbal grafts or limbal progenitor cells. Older donors (over 65 years) are often preferred for DMEK surgery as the graft in older donors is easier to unfold during surgery [14], although a clinical study does not suggest any clinical disadvantage of using donors younger than 55 years [15].

17.2.1.3. Malignancies

Haematological neoplasms, retinoblastoma and malignant tumours of the anterior segment are obligatory contraindications to cornea donation. Donors with certain malignant diseases may be evaluated and considered for avascular cornea donation but not for vascularised ocular tissues (i.e. limbal tissue, limbal stem cells or sclera). A report of metastatic cholangiocarcinoma cells found in the sclera and sclerocornea interface, but not avascular cornea, supports this [16]; but there has been a more recent report of malignant cutaneous melanoma (MCM) metastases in peripheral, but not central, avascular cornea [17]. The Medical Advisory Board of the Eye Bank Association of America has issued an amendment to their donor-selection criteria excluding donors with a history of melanoma with known metastatic disease [18]. The incidence of metastases from non-ocular tumours to the anterior segment of the eye is reportedly extremely low; however, corneas must be excluded where there is evidence of anterior segment metastases from the slitlamp examination of the eye or the corneoscleral disc [19, 20, 21]. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

17.2.1.4. Infections

Individuals with localised ocular infection (bacterial, viral, fungal, protozoal, parasitic) are excluded from donation of ocular tissues. This exclusion includes those with a history of past ocular *Herpes* infection. As these diseases typically are unilateral, only the affected eye must be excluded. The

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utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

Individuals suffering from bacterial septicaemia may be considered for cornea donation, provided that the corneas are stored by organ culture and the medium tested for microbial contamination before transplantation. Donors colonised with multidrug-resistant bacteria need a thorough risk assessment before they may be accepted as donors.

17.2.1.5. Diabetes mellitus

Diabetes mellitus may exclude cornea donation for EK, but not donation for DALK or PK. It increases the risk of unsuccessful DMEK graft preparation [22]. However, the risk of unsuccessful graft preparation may be related to the severity of the diabetes and an algorithm has been proposed to allow grafts to be prepared from corneas from donors with less severe disease [23].

17.2.1.6. Eye diseases

The following exclude cornea donation:

a. ocular inflammation and infection (see 17.2.1.4);
b. autoimmune disease, e.g. sarcoidosis, rheumatoid arthritis, but only where there is ocular involvement.

The following exclude cornea donation for PK or DALK, but not necessarily for EK:

a. corneal disorders including keratoconus, keratoglobus and epithelial and stromal dystrophies. As these diseases typically are bilateral, both eyes should be excluded;
b. corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central area of the cornea. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

17.2.1.7. Previous intraocular or anterior segment surgery

The following exclude cornea donation:

a. previous ocular surgery that would prejudice graft outcome;
b. receipt of a corneal, sclera or limbal allograft.

The following exclude cornea donation for PK or DALK, but not necessarily for EK:

a. refractive corneal surgical procedures, including radial keratotomy, lamellar inserts and laser refractive surgery. As these procedures typically are performed bilaterally, both eyes should be excluded.

17.3. Procurement

17.3.1. *Post mortem* time

Ocular tissues should be procured from donors as soon as possible after cardiac arrest, preferably within 24 h; however, Health Authorities or local practice may allow procurement up to 72 h after cardiac arrest. For EU member states, a blood sample for the mandatory tests for transmissible disease must be obtained from the donor within 24 h of death (see Chapter 5).

17.3.2. Procurement team

Ocular procurement personnel must be appropriately clothed and apply aseptic technique to minimise the risk of contamination of the tissue to be removed and also to protect personnel. Usually, this requires hand disinfection, the wearing of sterile gowns and gloves and the use of face masks or protective masks.

17.3.3. Procurement procedures

Since the ocular surface is exposed to the environment and, after death, there is no blinking and no tear film, the ocular surface is highly likely to be contaminated by environmental micro-organisms before procurement. Therefore, a classified area with a specified air quality is not typically required for ocular tissue procurement but other guidance given in Chapter 6 does apply. In case of concomitant skin donation, procurement of skin before ocular tissue is recommended (as described in §19.3.1).

The donor's eyelids and skin should be cleaned with an antiseptic solution and a sterile drape (eye sheet) placed over the face leaving the eyes exposed. The donor's eyes should be flushed with an appropriate sterile solution to remove debris, mucus and foreign matter from the cornea and conjunctival sac. An antiseptic solution suitable for cleaning the ocular surface prior to intraocular surgery may be applied to the ocular surface. A broad-spectrum antibiotic solution may also be used. After insertion of a lid speculum, peritomy is performed, preferably leaving a frill of conjunctiva at the limbus to avoid damage to the limbal progenitor cell niche.

The subsequent procedure depends on whether the eye is to be enucleated or just the corneoscleral disc procured by *in situ* excision. Advantages of *in situ* excision include: reduced death-to-preservation time since the corneoscleral disc is placed in storage medium immediately following procurement; potentially improved cosmetic reconstruction of the donor; and it may be more acceptable to some families than enucleation. However, procurement by enucleation is simpler, with less risk of harm to the corneal endothelium; and enucleated eyes provide sclera, for glaucoma or reconstructive surgery, and retina, optic nerve, lens and iris for research. There is no reported evidence of a difference in corneal quality or clinical outcome between these two procurement methods.

17.3.3.1. Procurement of the whole eye

The lateral extraocular muscle is located and lifted with a muscle hook and clamped with artery forceps close to its point of attachment to the sclera. The muscle is divided distally, leaving the artery forceps in place to stabilise and steady the eye. The remaining rectus muscles are then lifted in turn with the muscle hook and divided close to the sclera. It is not necessary to divide the oblique muscles. The eye is then gently lifted and the optic nerve severed using curved enucleation scissors. After enucleation, the eye should be placed, cornea uppermost, in a fixed position in a moist chamber and transported to the tissue establishment refrigerated in ice. Broad-spectrum antibiotics may be used to further minimise the risk of bacterial contamination.

17.3.3.2. Procurement of corneoscleral discs

After peritomy, sclerotomy is performed, maintaining a wide scleral rim (*circa* 4 mm) around the cornea. The corneoscleral disc is then gently lifted away from the eye without folding, to avoid damage to the endothelium. After excision, the corneoscleral disc should be immersed, endothelium uppermost to avoid the risk of damage, in an appropriate corneal storage solution that may contain antibiotics and antimycotics. Unless the cornea is to be transferred directly to organ culture at the tissue establishment, the container should either be a corneal viewing chamber or should have a flat bottom and adequate optical properties to facilitate subsequent assessment by slit lamp and specular microscopy.

17.3.3.3. Procurement of scleral tissue

Scleral tissue is prepared in the tissue establishment from the whole eye after excision of the corneoscleral disc.

17.3.4. Reconstruction of the donor

The aim is to mimic as closely as possible the original profile of the donor's closed eyes. After enucleation, the orbit should be filled with an appropriate prosthesis or other suitable material. The eye lids are then closed to restore the appearance of the donor.

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17.4. Temporary storage and transportation to the tissue establishment

Whole eyes should be stored and transported in a moist chamber at 2 to 8 °C. The time from procurement to processing at the tissue establishment should not exceed 24-48 h.

Corneoscleral discs procured by *in situ* excision may be placed in a hypothermic storage solution or in a medium designated by the manufacturer for room temperature storage. In both cases, the manufacturer's recommendations for storage temperature should be followed or the temperature conditions during transport should be validated.

17.5. Processing, preservation and storage

17.5.1. Processing facilities

The requirements of Chapter 8 and 9 on processing, preservation and storage should be applied when selecting an appropriate air-quality specification for ocular tissue processing and for environmental monitoring and quality control.

Table 17.1. Factors influencing the air quality for processing ocular tissue

Criterion	*Ocular tissue-specific*
Risk of contamination of tissues or cells during processing	Corneoscleral discs procured by *in situ* excision are placed in a storage medium in an environment where air quality typically is not controlled. Careful cleaning of the ocular surface before excision, aseptic technique and use of antimicrobials in the storage medium help to minimise the risk of contamination. Processing whole eyes in a tissue establishment allows control of air quality (e.g. laminar flow cabinet in a room with HEPA-filtered air). Cleaning of the eyes before processing is important because it has to be assumed that bacteria and fungi will be present on the ocular surface owing to lack of blinking and tear film after death of the donor. Organ-cultured corneas may be removed from their storage medium just prior to surgery to examine the endothelium by light microscopy and for further processing for EK. They are therefore re-exposed to the environment and an appropriate air quality must be applied. The EU Tissues and Cells Directive requires the equivalent of Grade A air quality with at least a Grade D background for such purposes.
Use of antimicrobials during processing	Corneoscleral discs may be stored in media containing antibiotics and antimycotics. The medium may also contain a marker (e.g. phenol red) that changes colour with a fall in pH caused by growth of micro-organisms. Turbidity of the storage medium is also an indication of contamination. Storage of corneas in organ culture not only allows the testing of samples of medium for microbial growth during storage but also ensures that any antimicrobials in the medium will be more effective, owing to the higher storage temperature than that used for hypothermic storage.
Risk that contaminants will not be detected in final tissue or cells due to limitations of the sampling method	There is typically no microbiological testing of hypothermic corneal storage media. Even if a sample of hypothermic medium is taken, the time available before transplant is limited to just a few days, which reduces the chance of detecting contaminants. Some eye banks recommend that surgeons send the remaining corneoscleral rim and storage medium for microbiological testing after preparation and transplantation of the corneal graft. For organ-cultured corneas, there is a greater chance of detecting contamination because of the extended, albeit still limited, storage period. A second sample of storage medium may be taken after transfer of an organ-cultured cornea to medium, to reverse stromal oedema and for transport to the recipient hospital, but the time before transplantation is only a few days and a negative-to-date release will apply. Therefore, there is a risk that contamination may not be detected until after transplantation.
Risk of transfer of contaminants at transplantation	Corneal tissue for the great majority of transplant procedures cannot be sterilised because living cells are required for a successful graft outcome. Post-operative endophthalmitis caused by micro-organisms transferred with the graft is therefore a risk and is a defined serious adverse reaction. It is considered to be rare. Attributing a cause is not always straightforward owing to the, albeit slight, risk of post-operative infection associated with any intraocular surgical procedure.

HEPA filter: high-efficiency particulate air filter.

17.5.2. Cornea processing methods

When corneoscleral discs have been procured by *in situ* excision, they will already be in a storage medium on arrival at a tissue establishment and may not require further processing unless they are to be

transferred to organ culture. When whole eyes are received by a tissue establishment, they should be subjected to a cleaning protocol to reduce the bioburden on the ocular surface before excision of the corneoscleral disc; for example, rinsing in sterile saline and immersion in a disinfectant such as povidone-iodine or chlorhexidine [24]. Further processing of corneoscleral discs to prepare grafts for EK may be undertaken in the tissue establishment [25, 26].

17.5.3. Cornea storage methods

a. Hypothermic storage at 2 to 8 °C

i. For whole eyes in moist chambers, storage times of $<$ 48 h are recommended for procedures where a viable corneal endothelium is required. This may be extended to 72 h for other purposes.

ii. For corneoscleral discs in storage medium, the manufacturer's recommendations should be followed for storage temperature and for maximum storage time, which can vary up to 21 days [27, 28, 29]. Corneas prepared in a tissue establishment for DSAEK or for DMEK can be shipped to hospitals in hypothermic storage media.

b. Organ culture at 28 to 37 °C

iii. A storage time of up to 4-5 weeks is typical for organ culture, although successful transplants after 7 weeks have been reported [30]. It is at the discretion of the Responsible Person (RP) or medical director to approve prolonged storage times, provided that the procedure has been validated. An inspection of the endothelium is mandatory at the end of the storage period and then the transplant can be assigned to the proper kind of surgery based on the cell density.
Renewal of the storage medium using aseptic procedures during the storage period is at the discretion of the RP/medical director and may depend on the manufacturer's recommendations.

iv. To reverse the stromal oedema that occurs during organ culture, corneas are transferred to a medium, the transport or 'deswelling' medium, containing a macromolecule to increase oncotic pressure and induce an efflux of water from the stroma. The cornea may be kept at 28 to 37 °C for up to 4-6 days, at the discretion of the RP and depending on the medium used [31, 32].

v. Organ-cultured corneas can be prepared in the eye bank for DSAEK after pre-thinning in dextran medium, or for DMEK with or without pre-thinning [33]. The DSAEK grafts may be laid back on the anterior stroma to provide additional support during transport in dextran medium [34]. DSAEK grafts are prepared after mounting the corneoscleral disc in a pressurised artificial anterior chamber followed by cutting away the anterior stroma using a microkeratome. Clinical results after graft preparation using a femtosecond laser have been found inferior when compared with microkeratome-cut DSAEK grafts [35, 36]. Clinical quality-control studies comparing DSAEK grafts prepared in an eye bank with DSAEK grafts prepared by the surgeon immediately before surgery have not identified differences in early complications (graft detachment), primary graft failure or endothelial cell density two years after surgery [37]. DSAEK grafts possibly should be cut as thin as possible because visual acuity is better after grafting with a thin graft compared with a thick graft [38].

vi. Of relevance for eye banks, DMEK grafts can be prepared by manual dissection, pneumatic dissection, or hydrodissection. A no-touch technique, without direct physical tissue manipulation during tissue preparation, may be an ideal approach to minimise graft damage [39]. Clinical results after eye-bank- and surgeon-prepared DMEK grafts seem similar [40]. For DMEK, the graft may be supplied rolled in the final diameter direct into medium or attached, either in the centre or at the periphery, and laid back on the stroma [26, 41]. Pre-prepared grafts for DSAEK and DMEK may be shipped to hospitals in medium at room temperature [26]. Pre-loaded graft for both DSAEK and DMEK can also be provided in order to minimise the time and efforts needed for tissue preparation in the theatre [42, 43].

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c. Storage of non-viable corneal tissue

vii. Corneoscleral discs or pieces of cornea for glaucoma or reconstructive surgery may be stored in ethanol (≥ 70 % v/v) or glycerol for extended periods. Corneal tissue may also be irradiated and stored in albumin, cryopreserved or frozen [44].

17.5.4. Sclera processing and storage

After excision of the corneoscleral disc from the eye, sclera is prepared using aseptic techniques by removing the intraocular contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). Sclera may be stored – whole, or divided into smaller, individually packaged pieces – in ethanol (≥ 70 % v/v) or glycerol, or fixed in formalin, freeze-dried, frozen or kept in saline with antibiotics. Sclera stored in saline with antibiotics in a refrigerator should only be kept for short periods (≤ 7 days).

17.6. Microbiological testing

Testing may be carried out before processing by swabbing the eye before excision of the corneoscleral disc; however, there appears to be little predictive value in this procedure [45, 46]. At the discretion of the transplanting surgeon, any corneoscleral tissue and storage medium remaining after preparation of the graft may be sent for microbiological testing; although there appears to be little predictive value from this [47], it can be helpful for the investigation of post-operative endophthalmitis. For further information refer to Chapter 10 on the principles of microbiological testing.

a. Organ-culture storage of corneas

Since corneoscleral discs intended for transplants requiring viable cells cannot be sterilised, microbiological testing of samples of organ-culture medium taken during corneal storage must be undertaken to test for microbial contamination. Microbiological media for bacteria and fungi should be inoculated and incubated at appropriate temperatures. A minimum corneal storage period of at least 3 days is required before taking samples for microbiological testing. In addition to microbiological testing, the culture medium should be inspected regularly for turbidity and change in pH (e.g. change in colour of phenol red in the medium), which may indicate microbial contamination. It is recommended to keep the organ-culture medium for at least a week after transfer of the corneoscleral disc to transport medium to allow additional monitoring for signs of contamination.

Further microbiological testing should, if possible, be carried out whenever a cornea is re-exposed to the environment, for example after endothelial assessment and transfer of the cornea into the transport medium or after preparation of corneas for DSAEK or DMEK in a tissue establishment. However, given the restricted time a cornea may remain in this medium (< 4-6 days), it is possible that growth of micro-organisms may not be detected before the cornea is transplanted. A negative-to-date release is possible, as described in Chapter 10. If growth is detected, the surgeon must be informed immediately to stop the tissue being transplanted. If the transplant has taken place, the identification and sensitivities of the contaminating micro-organisms must be established as soon as possible in order to help the surgeon's post-operative management of the recipient. The fellow cornea should be discarded. If the fellow cornea has already been transplanted, the transplanting surgeon should be informed and the patient monitored.

b. Hypothermic storage of corneas

Taking a sample of medium for microbiological testing during hypothermic storage of corneas is not standard practice, but may be required by national guidelines. Testing a sample of medium is a surrogate for direct testing of tissue. The short storage period and low temperature, which would suppress microbial growth, greatly reduce the likelihood of detection of contamination in the medium.

c. Sclera

Depending on the method of storage, for example refrigerated in saline, microbiological testing should be carried out after processing. Storage in ethanol (≥ 70 % v/v), glycerol (≥ 85 % v/v) or gamma

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8661 irradiation of the tissue may render microbiological testing unnecessary unless required by local or
8662 national guidelines.

8663 17.7. Quality control and cornea evaluation

8664 Quality-control tests on corneal grafts should consider at least the following minimum quality criteria:
8665 a. no evidence of microbiological growth (aerobic or anaerobic bacteria, yeast or fungi);
8666 b. endothelial characteristics;
8667 c. morphology and integrity of the cornea layers;
8668 d. diameter of clear central area of cornea.

8669 Depending on the specific use of the cornea, it is necessary to document the appearance of:
8670 a. epithelium, taking into account that the epithelium may partially detach or reduce in thickness
8671 during storage;
8672 b. stroma, which should have no central opacities or scars; the stroma of organ-cultured corneas may
8673 be hazy but should be transparent after reversal of stromal oedema in transport medium;
8674 c. endothelium.

8675 The quality-control tests to be carried out include the following:
8676 a. Gross examination
8677 i. abnormalities of the external globe;
8678 ii. signs of previous surgery of the anterior segment;
8679 iii. epithelial abrasions, retention of excessive orbital tissue or laceration of the globe;
8680 iv. epithelial defects;
8681 v. stromal opacities – a mild arcus senilis with a defined clear central zone may be acceptable;
8682 the minimal diameter of the clear zone is at the discretion of the RP/medical director;
8683 vi. abnormal corneal shape (keratoconus, micro- or megalocornea);
8684 vii. condition of the anterior chamber (shape, evidence of blood);
8685 viii. abnormalities, such as the pterygium extending over the optical zone of the cornea.
8686 b. Slit-lamp evaluation
8687 i. Slit-lamp examination of whole eyes and corneoscleral discs is recommended by the European
8688 Eye Bank Association [21].
8689 ii. It facilitates exclusion of pathological changes to the epithelium or stroma, such as scars,
8690 oedema, significant arcus, striae, epithelial defects, endothelial guttae or disease, infiltrates or
8691 foreign bodies, and anterior segment tumours or metastases.
8692 c. Microscopic evaluation of corneal endothelium
8693 i. The aim is to provide an estimate of endothelial cell density and a qualitative assessment of
8694 the appearance of the endothelium.
8695 ii. This evaluation must be applied to all corneas intended for PK or EK in order to minimise the
8696 risk that factors such as low endothelial cell density may have a negative influence on graft
8697 survival [12].
8698 iii. For corneas stored by hypothermia, this assessment is typically at the start of storage.
8699 iv. If the corneoscleral disc is not in a corneal viewing chamber, it needs to be turned over so that
8700 the endothelium is facing downwards to allow observation by specular microscopy through
8701 the base of the container.
8702 v. It should then be returned to the endothelium-uppermost position to avoid the risk of
8703 subsequent damage.
8704 vi. For organ-cultured corneas, this endothelial assessment can be both at the start and at the end
8705 of the storage period; assessment at the end of storage, shortly before the cornea is
8706 transplanted, is considered to be essential, whereas assessment at both the start and end of
8707 storage allows endothelial cell loss during storage to be determined.

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8708 There are two main methods used for endothelial evaluation by microscopy:
8709 vii. Specular microscopy. This method allows direct examination of the endothelium without
8710 staining; however, the appearance of the endothelial cells varies with temperature, type and
8711 time of preservation and the storage medium used. It is recommended that cold-stored corneas
8712 are warmed to room temperature to enhance the quality of the endothelial image.
8713 viii. Transmitted light microscopy (bright field or phase contrast). To enable cell counting, brief
8714 exposure to hypotonic sucrose solution (1.8 % w/v) or 4 minutes exposure to either balanced
8715 salt solution (BSS) or 0.9 % (w/v) NaCl is necessary to make endothelial cell borders visible.
8716 The exposure time to these solutions must be limited. Prior use of a stain such as trypan blue
8717 (0.06-0.4 %) will help to identify dead cells and areas of denuded Descemet membrane.

8718 Contraindications to use of corneas for PK or EK include:
8719 i. low endothelial cell density – the minimum endothelial cell density is set at the discretion of
8720 the RP/medical director but is typically 2 000 to 2 500 cells/mm²;
8721 ii. moderate to severe signs of polymegathism and pleomorphism;
8722 iii. significant (> 20 %) endothelial cell loss during organ culture;
8723 iv. abnormalities such as guttae;
8724 v. central stromal scars or opacities (may be acceptable for EK depending on cause and depth);
8725 vi. presence of dead endothelial cells – corneas with scattered, isolated dead cells are acceptable,
8726 whereas corneas with larger areas of dead or missing cells are not.

8727 For eye-bank-prepared tissues for DSAEK or DMEK, quality control also includes:
8728 a. gross inspection for larger variations in the thickness of DSAEK grafts and edge ruptures in
8729 DMEK grafts;
8730 b. measurement of the overall diameter of the prepared graft;
8731 c. for DSAEK grafts, measurement of the central thickness of the graft by ultrasound or optical
8732 coherence tomography.

8733 17.8. Corneal transplant registries

8734 Corneal transplant registries, such as those in Australia, the Netherlands, Sweden and the UK, provide
8735 an invaluable resource to validate the quality and safety of transplanted corneas. Registries also allow
8736 investigation of donor and recipient factors influencing graft survival, post-operative complications
8737 (including immunological rejection and serious adverse reactions) and visual outcome [4, 12, 48].
8738 Although randomised clinical trials (RCT) are considered to provide the highest level of evidence, they
8739 are costly and complicated to set up, they can be undermined by changes in clinical and surgical practice
8740 during the course of the study, and it is not always straightforward to generalise beyond the specific
8741 inclusion/exclusion criteria of an RCT, especially when corneal transplantation outcomes and risk of
8742 post-operative complications are influenced by many factors.
8743 Registries, while not without pitfalls, rely on large datasets to reduce selection bias. They provide
8744 a broad overview across multiple transplant units and an evidence base that does not always reflect the
8745 optimism generated by the excellent results from single-centre studies [49, 50]. In addition to evaluating
8746 the outcome of established techniques and monitoring the uptake and success of new processing and
8747 surgical techniques, such as endothelial keratoplasty, registry data can also be used for validating eye-
8748 bank processes and storage methods in terms of clinical outcome measures rather than simply relying
8749 on *in vitro* laboratory measures of quality and safety [12]. A project, part funded by the EU and led by
8750 the European Society for Cataract and Refractive Surgery, aims to establish a European Cornea and Cell
8751 Transplant Registry (ECCTR), building on the existing registries in the Netherlands, Sweden and the
8752 UK (see www.ecctr.org).

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8753 ## 17.9.Biovigilance

8754 Serious adverse reactions (SARs) for corneal transplants include:
8755 a. primary graft failure (corneal transplant never cleared);
8756 b. local infection (endophthalmitis or other serious ophthalmic infection);
8757 c. graft failure due to a defect in the donor tissue, which was out of date, scarred or marked by
8758 incisions from previous surgery);
8759 d. transmission of malignancy (possibly attributable to the transplanted tissue);
8760 e. transmission of systemic infection (possibly attributable to the transplanted tissue).

8761 Serious adverse events (SAEs) include:
8762 a. wrong tissue supplied for the intended surgical procedure;
8763 b. tissue supplied was damaged or showed signs of unacceptable previous surgery;
8764 c. tissue supplied beyond its expiry date;
8765 d. infection detected in organ-culture medium after cornea supplied to surgeon.

8766 Partial or complete graft detachment after EK is not uncommon; although the reported incidences
8767 vary, they are often in the range of 5-10% for DSAEK procedures and 10-30% for DMEK procedures
8768 [51]. Fortunately, graft detachment is most often successfully treated by re-bubbling (repeated air
8769 tamponade of the anterior chamber of the recipient). It has not been possible to ascribe this serious
8770 adverse event to donor factors such as donor endothelial cell density, donor age or method of
8771 preservation [52, 53]. Consequently, graft detachment is mainly considered a surgical complication.
8772 The Notify Library includes some well-documented cases of adverse reactions and adverse events
8773 in transplantation of ocular tissue; for example:
8774 • A case of donor-to-recipient transmission of the *Herpes simplex* virus (HSV) by cornea
8775 transplantation was confirmed by polymerase chain reaction-based DNA fingerprinting of
8776 donor and recipient HSV strains (Record Number 429);
8777 • A case of a transplant-acquired diagnosis of rabies is supported by temporal association of the
8778 recipient's illness, lack of other exposure to rabies and the retro-orbital pain of the recipient of
8779 the corneal transplant (Record Number 20);
8780 • A case of transmission of T-cell lymphoma is described, whereby molecular analyses were used
8781 to detect the same alleles in HLA-DQα testing of the recipient and donor of the graft (Record
8782 Number 338);
8783 • A case of donor ocular tissue being examined and then shipped to the eye bank with a contact
8784 lens on the cornea, thereby highlighting the importance of *in situ* inspection before recovery
8785 (Record Number 720);
8786 • A case of metastases from a cholangiocarcinoma in the vascularised limbal region of a
8787 corneoscleral disc. There was no evidence of transmission to the recipient of the avascular
8788 corneal graft. The authors recommended that tissue from donors with a history of malignancy
8789 should not be used for limbal allografting (Record Number 1663) [16].

8790 Further cases of adverse outcomes associated with ocular tissue can be found in the Notify Library
8791 at www.notifylibrary.org. The database is publicly accessible and can be searched by the substance type,
8792 adverse occurrence type and record number. A recent report, not yet in the Notify Library, concerns the
8793 identification of metastases in the peripheral, but not central, avascular cornea from a donor with
8794 malignant cutaneous melanoma (see 17.2.1.3) [17]. The implications for donor-selection criteria have
8795 been considered by the Eye Bank Association of America Medical Advisory Board, resulting in the
8796 exclusion of donors with a history of melanoma with known metastatic disease [18].
8797 Examples of two different SAR notification reports for ocular tissues used in France and by the
8798 NHS (UK) are included as appendices 25 and 26.

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17.10. Developing applications for patient treatment

The Bowman Layer lies between the epithelial basement membrane and the stroma. It can be dissected from donor corneas and inserted into the mid stroma of corneas with advanced keratoconus to help strengthen and flatten the patient's cornea [54].

Decellularised stroma can be used as a scaffold or for transplantation for corneal scars/ulcers. Decellularised porcine stroma is being used clinically, and development of human decellularised stroma is in progress [55]. Retinal pigment epithelial cells derived from human embryonic stem cells, induced pluripotent stem cells, umbilical cord, fetal brain or bone marrow are being investigated for the treatment of age-related macular degeneration (see Part C) [56].

Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease (see Part C) [9].

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8930 **Related documents**
8931 **Appendix 25: Notification form for SAR ocular tissues (Agence de la Biomédecine, France)**
8932 **Appendix 26: Notification form for SAR ocular tissues (NHS, UK)**
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Chapter 18. Amniotic membrane

18.1. Introduction

Amniotic membrane (AM) allografts have been used in different medical specialties since the early 20th century. Histologically, AM is the innermost, semi-transparent layer of the fetal membranes (amnion and chorion), formed by a single layer of cuboidal epithelial cells (epidermis-like cells), that is attached to a thick basement membrane and an avascular stromal matrix consisting of scattered fibroblasts in a collagen scaffold. The amnion contains no blood vessels, lymphatic vessels or nerves. It has some unique properties. A number of mechanisms have been suggested to explain the beneficial effects of AM, on the basis of its biological composition. Overall, AM is mainly formed by three types of components: structural collagen and extracellular matrix, biologically active cells and a large number of important regenerative molecules [1].

Clinical and experimental data have shown [2, 3, 4, 5, 6] that AM provides a compatible substrate for cell growth, facilitating migration and differentiation of epithelial cells, supporting maintenance of the original epithelial phenotype and having low or no antigenicity

18.1.1. Properties of amniotic membrane

Collagen types I, III, IV, V and VII and specialised proteins (including laminin and fibronectin) have been identified in the amniotic basement membrane and stroma. Laminin and fibronectin are particularly effective in facilitating epithelial cell adhesion. The presence of a rich extracellular matrix and collagen endows the stroma with anti-inflammatory properties, which arise from the entrapment of inflammatory cells, the presence of various growth factors and the inhibition of protease activity and decreased lipid peroxidation. In addition, AM has other biological properties that include anti-adhesive, antibacterial and anti-fibrosis effects, along with the ability to decrease scarring and neovascularisation, enhance wound healing and reduce pain [7, 8, 9]. Moreover, AM contains various growth factors (e.g. epidermal growth factor, basic fibroblast growth factor, hepatocyte growth factor, keratinocyte growth factor and transforming growth factors) and cytokines (e.g. interleukin 6 and 8) [10, 11, 12].

These characteristics have led to the use of AM for a wide range of ophthalmic indications (e. g. corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, chemical or thermal burns) and in the treatment of a broad variety of pathological conditions including management of burns (as a temporary or permanent wound dressing), repair of skin lesions of different aetiologies (e.g. vascular ulcers, epidermolysis bullosa, radiation burns), in arthroplasty and in intra-abdominal and reconstructive as well as oral and maxillofacial surgery [13, 14, 15, 16, 17].

In addition the special structure and biological properties of AM make it an important potential source for scaffold material [18].

Stem cells derived from AM have been demonstrated to display multilineage potential and immuno-modulatory properties [19, 20].

AM can be donated either separately from fetal membranes or together with placenta. In this chapter, when discussing donation, the term ‘placenta/fetal membranes’ is used to mean ‘not processed’, whereas ‘AM’ here means ‘processed in a tissue establishment’.

The following generic chapters (Part A) of this Guide apply to AM banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, risk management and validation (Chapter 2);

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c. Recruitment of living donors, identification and consent (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);
h. Processing (Chapter 8);
i. Storage and release (Chapter 9);
j. Principles of microbiological testing (Chapter 10);
k. Distribution and import/export (Chapter 11);
l. Organisations responsible for human application (Chapter 12);
m. Computerised systems (Chapter 13);
n. Coding, labelling and packaging (Chapter 14);
o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

This chapter defines the additional specific requirements for AM.

18.2. Donor recruitment and evaluation

Prior to full-term delivery, potential donors are approached to ascertain whether they would be willing to donate their placenta/fetal membranes (see Chapter 3). A trained nurse or healthcare professional will discuss the donation process and complete the consent and medical and behavioural lifestyle assessment. General criteria for donor evaluation are described in Chapter 4. The potential donor should be evaluated before giving birth and after full consent, also having been informed that donation will take place only if the delivery is without any complications.

Placenta/fetal membranes should be collected only from living donors, after a full-term pregnancy.

18.2.1. Specific exclusion criteria

In addition to the general exclusion criteria described in Chapter 4, there are some specific conditions that exclude placenta/fetal membranes donation. The diseases of the female genital tract or other diseases of the donor or unborn child that present a risk to the recipient include but are not limited to:

a. significant local bacterial, viral, parasitic or mycotic infection of the genital tract, especially amniotic infection syndrome;
b. (known) malformation of the unborn/newborn;
c. premature rupturing of membranes;
d. endometritis;
e. meconium ileus.

Individual tissue establishments may have additional exclusionary criteria.

18.3. Procurement

18.3.1. Procurement facility and procurement team

Donor placenta/fetal membranes are procured by medical staff at obstetrics units after caesarean section. AM could be contaminated by normal vaginal flora during vaginal delivery; therefore, procurement under aseptic conditions after elective caesarean section is to be preferred. If placenta/fetal membranes are procured during vaginal delivery, different sterilisation procedures [21, 22, 23] should be applied to the processed AM (e.g. sterilisation by gamma irradiation). Staff undertaking procurement

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9021 must be dressed appropriately for the procedure so as to minimise the risk of contamination of the
9022 procured tissue and any hazard to themselves. Further details can be found in Chapter 6.

9023 **18.3.2. Temporary storage and transportation to the tissue establishment**
9024 Placenta/fetal membranes should be stored at appropriate temperatures so as to maintain the
9025 structural and biological properties of AM.
9026 The storage and transport time of procured placenta/fetal membranes should be kept as short as
9027 possible (the recommended maximum time is 24 h) and a temperature of 2-8 °C should not be exceeded.
9028 If AM is processed within less than 2 h after the delivery, the placenta/fetal membranes may be
9029 transported at room temperature [24].
9030 Procured placenta/fetal membranes should be placed in a sterile receptacle containing a suitable
9031 transport medium (or decontamination solution) if transport time exceeds 2 h [25]. The sterile packaging
9032 should then be placed inside an adequately labelled sterile container to be transported to the tissue
9033 establishment. Individual tissue establishments should validate the composition of the transport medium
9034 and determine if antibiotics are required.
9035 The temperature during transport to the tissue establishment must be maintained. Temperature
9036 stability should be guaranteed by the container, conditions of transport used and for the time interval
9037 before processing. In cases of unexpectedly high or low environmental temperatures, a temperature-
9038 recording unit (data logger) should be enclosed in the container to record temperature at ≤ 30-minute
9039 intervals unless the transport system has been previously validated to maintain the temperature within
9040 the required limits for the required transport time.

9041 ## 18.4.Processing and storage

9042 **18.4.1. Receipt of placenta/fetal membranes at the tissue establishment**
9043 Upon receipt, the procured tissue should be stored in a temperature-controlled refrigerator at 2-
9044 8 °C to ensure that the refrigeration process is not interrupted. Processing should be carried out within
9045 24 h after procurement [25].

9046 **18.4.2. Processing facilities**
9047 In selecting an appropriate air-quality specification for AM processing, the criteria identified in
9048 Chapters 7, 8 and 10 should be considered. Table 18.1 outlines factors to be considered for AM
9049 processing.
9050 Taking the factors from Table 18.1 into consideration, it is appropriate that processing of AM
9051 should take place in a controlled environment with defined air quality (see Chapter 7), especially for
9052 cryoprotected AM where there is less opportunity for microbial inactivation (Grade A environment with
9053 Grade B background is recommended, to ensure aseptic processing of cryoprotected AM allografts).
9054 Within the EU, tissues exposed to the environment without subsequent microbial inactivation
9055 should be processed in environments with an air quality equivalent to GMP Grade A, with a background
9056 environment of at least Grade D.
9057
9058

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9059 **Table 18.1. Factors influencing the air-quality specification for processing amniotic membrane**

Criterion	*Amnion-specific*
Risk of contamination of tissues or cells during processing	During processing, AM is necessarily exposed to the processing environment for extended periods during dissection, sizing and evaluation of its characteristics.
Use of antimicrobials during processing	Soaking in antibiotic/antimycotic solution is an antimicrobial step that can be used when processing AMs. It is important to validate the decontamination solution and to list the micro-organisms that are acceptable pre-decontamination. Since glycerolised, lyophilised and frozen AM can be exposed to sterilisation processes, the processing environment may not be as critical as for tissue that cannot be sterilised. However, the process should be validated, and maximal acceptable bioburden defined.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of AM for microbiological testing following decontamination step is not extensive; typically, only a small amount is sampled, but the storage medium can also be sampled.
Risk of transfer of contaminants at transplantation	Although not vascularised, AM can support microbiological contaminants and has transmitted bacteria and viruses. AM is mostly used in ophthalmology. AM is also used for other indications, such as burns, skin ulcers and arthroplasty, and intra-abdominal and reconstructive as well as oral and maxillofacial surgery. Immuno-compromised patients, despite recent advances in therapy, are at a substantially higher risk of transmission of infection and even death from infections.

9060

9061 **18.4.3. Processing and preservation methods**

9062 Processing must not change the physical and biological properties of AM so as to make it
9063 unacceptable for clinical use. Tissue establishments may use different processing and preservation
9064 methods, according to their own standard operating procedures (SOPs) and mandatory regulations. The
9065 methods used must be in line with current best practice and must be validated in accordance with the
9066 guidance given in Chapters 2 and 8.

9067 Processing of AM generally includes the mechanical detachment of fetal membranes (after being
9068 previously separated from placenta, when applicable), according to a documented SOP. Fetal
9069 membranes easily split into an amnion and a chorion leaflet, separated by a jelly-like, intermediate layer.
9070 The chorion is discarded and then the amnion should be rinsed several times in sterile saline until blood
9071 residues are removed completely. During processing, AM may be decontaminated by soaking in
9072 antibiotic/antimycotic solution. The incubation temperature and the composition of decontamination
9073 cocktails should be defined, after validation, by each tissue establishment. Following the
9074 decontamination step and rinsing procedure, AM should be spread on a suitable carrier membrane (e.g.
9075 nitrocellulose), or fine mesh gauze for easier handling and if it needs to be cut into multiple pieces.
9076 Depending on the intended clinical use, both sides of amnion (epithelial and mesenchymal) can be
9077 placed directly on the selected carrier.

9078 Consistent with the defined preservation method, AM grafts may be decontaminated or sterilised
9079 by irradiation. Sterilisation methods should be validated for the initial estimated level of bioburden [21,
9080 22, 23]. AM grafts should be packaged in sterile containers and labelled as advised in Chapter 14.
9081 Maximum storage time will depend on the preservation method and should be defined and validated
9082 [24, 26]. There are several methods of AM preparation and preservation, as below.

9083 *18.4.3.1. Cryoprotected amniotic membrane*

9084 AM can be preserved in culture medium containing glycerol or dimethyl sulphoxide (used to
9085 protect cells against freezing injury). AM intended to be cryoprotected may be decontaminated by
9086 soaking in antibiotic/antimycotic solution.

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9087 Following package, AM grafts are stored at −80 °C (deep frozen) or in liquid or vapour phase of
9088 nitrogen at temperatures below −140 ºC (cryopreserved) [27, 28, 29] but, in the case of cryopreservation,
9089 after being previously submitted to a controlled-rate freezing procedure.

9090 *18.4.3.2. Frozen amniotic membrane*

9091 If cell viability is not to be maintained, the processed AM can be frozen without addition of a
9092 cryoprotective agent.
9093 AM intended to be frozen may be decontaminated by antimicrobial solution or sterilised by
9094 irradiation [24]. Following package, AM grafts should be stored between −15 °C and −80 °C.

9095 *18.4.3.3. Heat-dried amniotic membrane*

9096 The processed AM is dried overnight in an oven at 40 ± 2 °C, then packed and sterilised by
9097 irradiation. Storage should be at room temperature [25].

9098 *18.4.3.4. Air-dried amniotic membrane*

9099 The processed AM is air-dried overnight in a laminar-flow hood. It can then be packed and
9100 sterilised by irradiation. Although high temperatures are not applied using this method, some properties
9101 of the amnion are lost or altered due to dehydration. Air-dried irradiated AM grafts should be stored at
9102 room temperature [30, 31].

9103 *18.4.3.5. Lyophilised (freeze-dried) amniotic membrane*

9104 The processed AM is rapidly frozen at −50 °C to −80 °C. Then it is vacuum-dried using a freeze-
9105 drying device. Water from the tissue is extracted through sublimation until a final water content of 5-
9106 10 % is attained. Following package, AM grafts may be sterilised by irradiation [32]. This preservation
9107 method induces minimal changes in the properties of the AM and the product can be stored at room
9108 temperature [33].

9109 *18.4.3.6. Glycerolised amniotic membrane*

9110 Glycerolisation is a preservation method combined with the antimicrobial properties of high
9111 concentrations of glycerol. Since glycerol permeates slower than water, there will be an initial efflux of
9112 water when the glycerol is added. However, as glycerol begins to permeate the tissue, water will re-
9113 enter. At the end of the glycerolisation process, the final water activity (a_w) is *circa* 0.3, which is known
9114 to minimise lipid peroxidation and reduce other degradation reaction rates to very low levels. Rather
9115 than dehydrating the tissue, as is commonly assumed, it has been demonstrated with skin that
9116 glycerolisation results in the effective sequestration of water [34, 35]. Typically, 85 % (v/v) glycerol is
9117 used to preserved AM, which can then be stored at 2-8 °C for up to two years, although it does lose some
9118 of its biological properties [36].
9119 AM intended to be glycerolised may be decontaminated by soaking in antimicrobial solution or
9120 sterilised by irradiation.

9121 *18.4.3.7. Antibiotic-soaked amniotic membrane*

9122 The processed AM is placed overnight in a decontamination solution composed of a range of
9123 wide-spectrum antibiotics and an anti-fungal agent and then deep-frozen at −80 °C. The resultant AM is
9124 particularly suitable for wound healing.
9125

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9126 **Table 18.2. Microbial contaminants that should result in tissue discard if detected at any stage of processing**

Acinetobacter spp.
Aspergillus spp.
Bacillus spp.
Bacteroides spp.
Beta-haemolytic *Streptococci*
Burkholderia cepacia complex
Candida spp.
Clostridium spp. (notably *C. perfringens*)
Corynebacterium diphtheriae
Enterobacteriaceae (coliforms)
Enterococcus spp.
Fusobacterium spp.
Klebsiella rhinoscleromatis
Listeria monocytogenes
Mucor spp.
Mycobacteria spp. (for at-risk donors)
Neisseria gonorrhoea
Nocardia spp.
Penicillium spp.
Pseudomonas spp.
Porphyromonas spp.
Prevotella spp.
Salmonella spp.
Shigella spp.
Staphylococcus aureus
Sphingomonas maltophilia
Stenotrophomonas maltophilia
Streptococcus pyogenes (Group A)
Other yeasts and fungi

Note: *This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.*

9127
18.5. Quality control

9128 During procurement and processing of AM, reliable macroscopic examination of the donor fetal
9129 membranes should be undertaken to exclude visible pathological changes and ensure structural integrity
9130 of the tissue (to provide barrier function). Samples for detecting aerobic and anaerobic bacteria and fungi
9131 should be obtained from the transport/storage medium or from the initial washings of the AM, and from
9132 pieces of the tissue obtained both before and after antibiotic decontamination step. Microbiological
9133 testing for the detection of bacteria and fungi should be carried out according to the procedures described
9134 in Chapter 10.
9135 These approaches cover the minimum standards to control microbiological safety (see **Table
9136 10.2**). Deviations from such standards should be justified, and the suitability of the intended test method
9137 must be demonstrated. Factors such as samples containing antibiotics or very small sample amounts
9138 may affect the sensitivity of tests leading, in the worst case, to false-negative results. Where samples
9139 taken before antibiotic/antimycotic decontamination yield micro-organisms that are considered
9140 pathogenic and highly virulent (see Table 18.2), the tissue cannot be approved for clinical use. Tissue
9141 showing heavy or confluent bioburden growth should also be rejected. After decontamination, tissue is
9142 not deemed suitable for transplantation if the samples taken for microbiological testing show signs of
9143 any microbial growth.

9144
18.6. Distribution

9145 The tissue establishment must ensure that distribution of AM grafts is carried out under controlled
9146 conditions. General considerations can be found in Chapter 11.

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For cryopreserved AM, distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. For deep-frozen AM grafts, distribution should be in dry ice. Transport temperatures of cryoprotected AM above −60 °C must be avoided, to ensure the stability of the product and maximum safety for the recipient. Frozen AM grafts should be transported at or below −15 °C.

Heat-dried, air-dried and freeze-dried AMs can be distributed at room temperature, whereas glycerolised AM should be transported at 2-8 °C.

18.7.Biovigilance

Serious adverse events and reactions must be recorded, reported and investigated according to the relevant national regulations to Health Authorities for tissues and cells, as described in Chapter 16.

The Notify Library includes some documented cases of adverse occurrences. Examples of SARs include:

- Loss of significant quantity of AM grafts due to storage at inadequate temperature (e.g. in a case of equipment failure).
- High level of microbial contamination of procured AM (reflecting the hygienic conditions of the delivery room).

18.8.Developing applications for patient treatment

18.8.1.1. Amniotic drops/suspension/extract

The use of drops/extracts described in the literature [37-48] shows that this form of application also has a good effect and can be used in many therapeutic fields as an alternative to transplantation.

18.8.1.2. Chorion

So far, literature hardly differentiates between amnion and chorion grafts, investigating fetal membranes in general. However, there are differences in composition between their membrane components [49], so that different effects from amniotic and chorionic membranes can be expected [50].

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Chapter 19. Skin

19.1. Introduction

Autologous skin is considered the gold standard for wound treatment and final wound closure. For large wounds, autologous thin split skin can be widely expanded (meshed) or transplanted as island grafts (Meek wall technique). The autologous skin grafts will grow out to close the wound.

In case of shortage of autologous skin sources (especially in burns), donor skin is used to treat patients with extensive skin loss, for wounds with either split-thickness or full-thickness depth. Deep injuries lead to dermal damage, impairing the ability of the skin to heal and regenerate. Skin allograft basically acts as a temporary coverage, preparing the wound bed, reducing scarring, controlling pain, preventing infection and maintaining patient homoeostasis by reducing loss of fluids, proteins and heat through the burn wound. For these reasons, donor skin is critical (and often life-saving) in the treatment of severely burned patients, after escharectomy. In addition, allogeneic skin is considered to be an excellent biological dressing for the treatment of other types of skin defects such as venous ulcers, decubitus ulcers, diabetic foot, surgical wounds, exfoliative skin disorders such as toxic epidermal necrolysis (Lyell's syndrome) and congenital epidermolytic skin disease. In these cases, skin allografts promote re-epithelialisation and formation of granulation tissue, shorten healing time, control pain and protect important structures (e.g. tendons, bones, cartilage, nerves) [1]; they are also successfully used as skin substitutes that incorporate the dermal component into the wound bed, guiding a more physiological healing process, having the ability to be integrated into the wound bed of full-thickness burns or leg ulcers.

Allogeneic skin can also be placed on top of the autologous skin (the 'sandwich technique') to protect it from mechanical damage, dehydration and infection. After 7-10 days the allogeneic skin can be removed from the wound [2, 3, 4].

These factors explain the constant demand for skin allografts by burn centres and reconstructive surgery units, where the capacity of these bio-products to 'take' and integrate into the wound bed is exploited. In the past, allogeneic skin was used sometimes to replace the lost dermis ('Cuono technique') [5], but the donor cells and hairs still present may cause inflammatory reactions with a negative effect on the final scar formation. Nowadays acellular dermis is available. Several tissue banks have developed this type of skin graft that is more suitable as a dermal equivalent.

The shortage of allogeneic skin grafts has promoted the development of skin-replacement products, and many research teams have focused on biomaterials for skin substitution in wound healing. In the past 30 years, a huge number of biological, semisynthetic and synthetic skin/dermal substitutes have been developed with the aim of producing an artificial skin that is able to replace human skin completely, but an ideal skin substitute has not yet been realised. A further logical development of this research involves the use of stem cells to re-populate the dermal matrix and reproduce 'physiological' skin, but to date there is no ideal skin-replacement product available based on stem cells.

The following generic chapters of this Guide (see Part A) all apply to skin banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, validation and risk management and (Chapter 2);
c. Recruitment of potential donors, identification and consent (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);

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h. Processing (Chapter 8);
i. Storage and release (Chapter 9);
j. Principles of microbiological testing (Chapter 10);
k. Distribution and import/export (Chapter 11);
l. Organisations responsible for human application (Chapter 12);
m. Computerised systems (Chapter 13);
n. Coding, labelling and packaging (Chapter 14);
o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

This chapter defines the additional specific requirements for skin.

19.2. Skin-specific donor evaluation

19.2.1. Skin inspection and skin-specific contraindications

In addition to the standard physical examination described in Chapter 4, the donor's skin must be inspected in a particular manner before skin procurement. Skin should be visually checked for mechanical damage, open wounds, multiple (> 100) or dysplastic naevi (see Appendix 16), dermatitis, local infections, scars and ectoparasites. The results must be recorded and taken into account.

The list of selection criteria for donors is based on a risk analysis related to the use of the tissue on patients, i.e. to minimise the risk of transfer of diseases to the recipient and to ensure the appropriate quality of skin for optimal functional results. The following conditions contraindicate skin donation:

a. autoimmune diseases and systemic connective tissue diseases affecting skin;
b. diseases affecting the dermis (e.g. dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria, lupus erythematosus);
c. toxicity of the skin as a result of the presence of toxic agents or poisons;
d. systemic use of corticosteroids or Cushing disease, inducing severe skin atrophy.

The following relative contraindications for skin donation should be considered case-by-case, and eventually require risk assessment:

a. extensive lacerations, haematoma or scars;
b. skin diseases with extensive involvement (e.g. psoriasis, eczema);
c. relevant skin ulcers, pressure ulcers, stoma, pyoderma or mycoses;
d. skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos, jaundice);
e. diabetes mellitus with skin complications (e.g. ulcers, amputation, neuropathy);
f. pre-malignant conditions such as actinic keratoses and Bowen's disease;
g. mechanical, thermal or microbial damage where skin is to be procured;
h. extreme peripheral oedema, high body mass index (BMI) or poor nutritional status affecting procurement or body reconstruction.

The common practice is not to procure skin from donors aged < 15 years but many tissue establishments do not indicate any age limits, which are basically determined by the medical director of the tissue establishment, according to characteristics and quality of tissues.

19.3. Skin procurement

Skin can be obtained from deceased donors after brain death (DBD) or circulatory death (DCD). It is recommended to procure the skin within a period of 12 h, should the body not be refrigerated, or up to 24 h after death if the body has been cooled or refrigerated within 6 h of death. It is obvious that

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refrigeration of the DCD donor before procurement will reduce skin contamination and facilitate skin procurement due to the harder consistency of the subcutaneous tissue.

It may be possible to extend procurement times up to 48 h after death if skin processing has been validated to guarantee quality and microbiological safety; in these cases the blood samples for serological testing should be taken within 24 h after death (to avoid extensive haemolysis) [6]. See Chapter 5 for details on sample collection.

Skin can also be obtained from living donors if there is a shortage of *post-mortem* donors, from patients having abdominoplasty or mammoplasty procedures who consent to tissue donation. Potential living donors are evaluated similarly to deceased donors to determine donor suitability according to standard exclusion criteria for skin donation and absence of adverse physical, psychological or emotional outcome before, during or following the donation (see Chapter 4). In these cases, the procurement area is prepared by depilation and disinfection and the tissue is processed to obtain full-thickness skin grafts.

19.3.1. Procurement team

Skin-procurement teams should consist of at least two people operating under aseptic conditions and appropriately clothed for the type of procurement. In the case of multiple-tissue procurements, the order in which the tissues are removed should be standardised and predefined and, in the case of multiple procurement teams, should be agreed between the teams beforehand so that risks of cross-contamination between tissues are minimised (see Chapter 6). Studies show that, whether the skin procurement is done before or after bone procurement, the contamination rate of skin is not different if the procurement process is controlled and standardised [7, 8]; therefore, skin is usually retrieved in aseptic technique prior to bone tissue due to the difficulty in obtaining grafts of consistent quality in particular after extensive bone procurement.

Notably, procurement of skin before ocular tissue is recommended to avoid eye bleeding from the sockets if the donor has to be placed in a prone position following enucleation of the eyes.

19.3.2. Skin-procurement procedure

Skin is procured under aseptic conditions after adequate shaving of the donor areas and appropriate pre-operative scrubbing and disinfection of the donor skin to remove the transient, and reduce the resident, microbial flora. An effective and validated procedure for skin disinfection should be established by the tissue establishment and allocated to all procurement sites.

The procedure should aim to reduce the bioburden, which can significantly decrease the microbial positivity rate of processed skin samples. Therefore, suitable disinfectants, such as povidone iodine or chlorhexidine, should be chosen. Their concentrations and the durations of exposure should also be evaluated and validated.

A local sterile field using sterile drapes must be used prior to procurement to effectively prevent microbial contamination. Skin grafts can be procured by manually, electrically, compressed-air or battery-operated dermatomes-from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs. Grafts should be cut as homogeneously as possible.

According to graft thickness, skin grafts can be divided into split-thickness and full-thickness grafts. They consist of the entire epidermis and a dermal component of variable thickness. If the entire thickness of the dermis and skin adnexal structures is included, the appropriate term is full-thickness skin graft (FTSG). This type of skin graft can be procured using a scalpel instead of a dermatome. If less than the entire thickness of the dermis is included, this graft is referred to as a split-thickness skin graft (STSG). STSGs are categorised further as thin (0.008-0.012 in/ 0.2-0.3 mm), medium (0.012-0.018 in/0.3-0.45 mm) or thick (0.018-0.030 in/0.45-0.75 mm). The choice between full- and split-thickness grafting (see Figure 19.1) depends on wound conditions, location, thickness, size and aesthetic concerns [9, 10].

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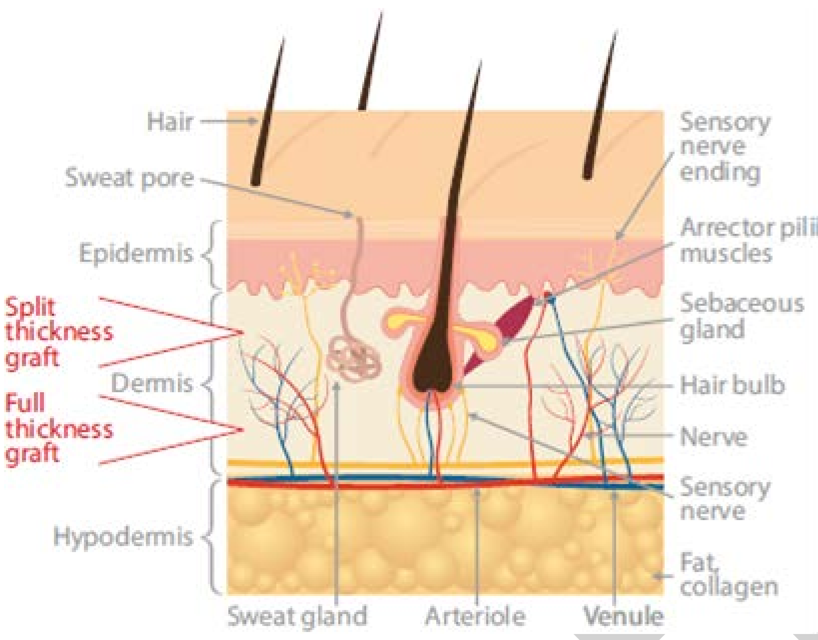

9413 **Figure 19.1. Differences between split- and full-thickness grafts**

9414
9415 Staged separate procurement of particular body areas, with placement of procured material from
9416 each area into separate containers, is sometimes preferred in order to reduce eventual cross-
9417 contamination of the procured tissue. Containers and solutions for transportation of procured skin to
9418 processing tissue establishments must be sterile and suitable for the intended use. Pre-labelling of the
9419 containers is important to prevent mix-up of tissues and to ensure their full traceability (see Chapter 14).

9420 **19.3.3. Reconstruction of the skin donor**
9421 For aesthetic reasons and with a view to a respectful reconstruction of the donor, it is not
9422 acceptable to take skin from the neck, face and other typically exposed areas of the body that might be
9423 visible when people pay their last respects to the donor. Conditions which may affect body
9424 reconstruction after procurement such as extreme oedema or a high BMI (≥ 40) should be evaluated
9425 prior to procurement; body reconstruction should ensure that loss of fluid that accompanied skin
9426 procurement is not noticeable. Once the tissue has been procured, sealing agents (polymers) and
9427 appropriate garments (e.g. polyethylene overalls) should be used to prevent leaking and oozing from
9428 sites where tissue has been obtained. Effective communication with all parties involved can help to meet
9429 expectations in regard to delays, as well as aesthetic considerations in case of unexpected leaking.

9430 **19.3.4. Temporary storage and transportation to the tissue establishment**
9431 Immediately after procurement, skin samples for initial bioburden estimation should be obtained
9432 and the recovered tissue must be stored in a suitable transport medium in sterile, pre-labelled containers
9433 filled with an appropriate transport medium. The containers must be sealed securely, refrigerated to 2-
9434 8°C and transported to the processing facility or tissue establishment. Transportation at low temperatures
9435 prevents proliferation of most bacteria and fungi, and maintains skin viability (if viable grafts are
9436 requested). Antibiotics can be added to the transport medium, but at 2-8°C even the latest broad-
9437 spectrum antibiotic cocktails can fail to decontaminate skin grafts. According to Rooney *et al.* [11],
9438 approximately 22% of skin allografts are not reliably decontaminated by antibiotic treatment.
9439 There are three likely causes as to why antibiotic cocktails are not always fully efficient. First of
9440 all, bacteria can be 'hidden' in the procured skin (e.g. in the hair follicles) where the antibiotics cannot
9441 reach them. Secondly, the optimal operating temperature of most antibiotics is much closer to 37°C than

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9442 to 2-8°C [12]. The inclusion of an additional short antibiotic incubation step at 37°C could be considered
9443 during processing stages. Finally, diluted antibiotic suspensions are known to lose activity relatively
9444 quickly (even when frozen). It is thus important that concentrated antibiotic suspensions are kept at low
9445 temperatures (e.g. according to manufacturer instructions) and are not added to the transport media much
9446 in advance.
9447 The container with procured skin must be cooled during transport to the tissue establishment. If
9448 skin grafts are to be glycerolised (i.e. they are non-viable grafts), refrigerated transportation may not be
9449 required and the recovered skin can be stored and transported at ambient temperature in 50% glycerol
9450 solution.
9451 After procurement, skin grafts should be transferred to the processing tissue establishment as soon
9452 as possible, and tissue processing should commence within 24-72 h of procurement having taken place.
9453 Before processing, the recovered skin should be kept in a temperature-controlled refrigerator at 2-8°C,
9454 without interruption throughout the refrigeration process. It is recommended that the cell nutrient
9455 medium used for viable grafts be changed shortly after receipt of skin grafts, or that the medium be
9456 validated for 72 h storage (i.e. adequate buffering capacity). All the manipulations where the transport
9457 containers are going to be exposed and the media changed must be performed in a controlled-air-
9458 environment safety cabinet in a clean room of at least Grade D.

9459 **19.3.5. Procurement documentation**

9460 The organisation responsible for the skin procurement must produce a procurement report to be
9461 provided to the tissue establishment. In addition to the generic requirements defined in Chapter 6, it
9462 must contain a description and identification of the procured skin (including samples for testing).

9463 ## 19.4. Skin processing

9464 The recovered skin is processed to reduce microbial contamination and allow longer storage periods
9465 until transplantation. All human tissues intended for human application are processed into specimens
9466 appropriate for clinical use. Processing must not change the physical properties of the tissue, making it
9467 unacceptable for clinical use.The methods used must be in line with current state-of-the-art procedures
9468 and validated procedures (see Chapters 2 and 8). Different tissue establishments apply specific
9469 preparation methods according to their own standard operating procedures (SOPs) and any applicable
9470 local authorisations. All processes must be validated in accordance with the guidance given in Chapter 2.

9471 **19.4.1. Skin processing methods**

9472 Depending on the intended clinical use and the quality requirements, skin grafts can be processed
9473 and preserved according to various methods (cryopreservation, glycerol preservation, lyophilisation,
9474 possibly followed by gamma-irradiation). These methods ascertain different grade of skin viability,
9475 integrity and immunogenicity. Skin grafts destined for cryopreservation should be processed
9476 immediately after receipt in order to maintain cell viability and structural integrity. Skin allografts can
9477 also be processed into de-epidermised skin and acellular dermis. Processing of skin grafts generally
9478 includes soaking in antibiotic and antimycotic cocktails; if skin viability is to be maintained, then this is
9479 the only decontamination step that can be included and it should always be applied. The incubation
9480 temperature and the composition of decontamination cocktails should be defined, after validation, in
9481 written procedures by each tissue establishment, assessing the initial tissue bioburden.
9482 The usual width of the grafts procured depends on the width of the dermatome blades (usually 8
9483 or 10 cm). The length of the grafts varies according to the size of the donor site and the final storage
9484 containers. Procured skin allografts can be cut into specific smaller sizes according to requirements of
9485 the end-user clinicians. The skin grafts may be provided as sheets or meshed (extended on a synthetic
9486 mesh to increase the surface area and to allow wound fluid drainage). The graft's irregular edges should
9487 be trimmed and, typically, a rectangular shape should be obtained. The final graft sizes are measured

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9488 with a ruler or calipers. The dimensions and area of each graft must be recorded and displayed on the
9489 label. The grafts should then be packaged in validated sterile packages and labelled appropriately (see
9490 Chapter 14).

9491 *19.4.1.1. Glycerol-preserved skin allografts*

9492 Glycerol-preserved skin allografts (GPA) were developed [13] to maintain skin allograft at 2-8 °C
9493 using an increasing series of glycerol concentrations (50 %, 70 %, 85 %) for preservation and storage
9494 without freezing. Glycerol preservation is an excellent preservation method to obtain de-vitalised skin
9495 grafts characterised by reduced immunogenicity and low antibacterial/antiviral properties [14-24]. If
9496 there are positive microbiology results from cryopreserved skin, the skin can be processed in 85 %
9497 glycerol as a recovery procedure. The glycerol solutions used must be sterile and of high quality (e.g.
9498 see *European Pharmacopoeia* monograph 0497 – Glycerol 85 %). Most skin banks validate an expiry
9499 date of 5 years for GPA stored at 2-8ºC.

9500 *19.4.1.2. Unprocessed skin allografts*

9501 The use of unprocessed skin allografts ('fresh skin allografts') is not the preferred option because
9502 it may not allow for complete donor screening, autopsy reports and/or extensive microbiological testing.
9503 However, some tissue establishments use unprocessed skin allografts as it is possible to maintain
9504 structural integrity and cell viability for short periods of time (maximum 7-8 days) [11]. These allografts
9505 were initially preferred in burn centres due to their high cell viability [25].

9506 *19.4.1.3. Cryopreserved and deep-frozen skin allografts*

9507 These preservation methods aim to maintain cell viability and structural integrity of skin
9508 allografts. Biological and structural functions of skin tissue are preserved by cooling to subzero
9509 temperatures in a freezing medium with cryoprotectants, such as dimethylsulphoxide (DMSO) or
9510 glycerol [26, 27], to protect cells against freezing injury. Cryoprotectants can, however, adversely affect
9511 cell viability and graft efficacy. A controlled-rate freezing procedure is recommended to preserve cell
9512 viability. Cell viability is maintained by cryopreserved and, to a lesser degree, deep-frozen skin grafts.
9513 It favours the tissue engraftment to the wound bed, being a substrate for revascularisation and
9514 recolonisation by host cells [28-29, 12]. Skin-cell viability, referred to as the mean percentage of cell
9515 viability after 10-20 days of storage, is reported to be between 40 and 50 % that of the fresh skin [30-33].
9516 When cell viability is required for clinical use, it should be validated and can be assessed by various
9517 methods, including vital dye staining, oxygen consumption, and enzymatic and metabolic assays
9518 (described in §19.5.2).
9519 Cryopreservation is considered to be the best method for the long-term preservation of skin [12].
9520 After cryopreservation, skin allografts can be stored in liquid or vapour nitrogen to a maximum of 5
9521 years. Storage at higher temperature (− 60 °C/− 80 °C) is a method applied for medium-term (maximum
9522 of 2 years) preservation of viable skin allografts. Inappropriate storage compromises the potential to
9523 restore normal metabolic activity and, thus, physiological functioning after transplantation.

9524 *19.4.1.4. Lyophilised skin allografts*

9525 Processing of grafts by freeze-drying devitalises the grafts while maintaining their structure. A
9526 maximum limit for residual water content should be established and measured (ideally < 5 %).
9527 Lyophilised skin grafts can be stored at ambient temperature for 3-5 years.

9528 *19.4.1.5. De-epidermised skin and acellular dermis*

9529 De-epidermising or de-cellularising skin is a method to lower the antigenicity of the skin graft.
9530 Thicker skin obtained from deceased donors is processed aseptically to remove the epidermis and
9531 possibly the dermal cells that can accelerate tissue rejection and graft failure. Acellular dermis can
9532 permanently replace the lost dermis in patients with full-thickness wounds (burns). Various methods for
9533 separating the epidermis from dermis are reported such as chemical (sodium chloride, phosphate
9534 buffered saline, dispase), physical (heat; freezing and thawing) or mechanical (dermatome). These
9535 methods are frequently used in association to obtain optimal de-epidermisation. In cases of shortage of

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deceased skin donors, full-thickness skin can be obtained from living donors undergoing abdominoplasty or body-contouring procedures and is processed in a similar manner to produce thicker dermal allografts to be used in full-thickness skin loss if primary closure or donor-site availability of autografts is limited or suboptimal [34]. The result is an intact dermal matrix that can be cryopreserved, preserved in glycerol or lyophilised.

19.4.1.6. Skin tissue decellularisation

Tissue decellularisation is a technique that aims to remove all cells from a tissue, maintaining an intact extracellular matrix (ECM). In the last years several innovative biological products based on decellularisation of donor-derived skin tissue have been developed using biotechnological sciences.

Three methods are actually used to obtain tissue decellularisation: chemical, physical and biological (enzymatic). Each of these methods has a different mode of action and effect on the ECM. A combination of methods is recommended to ensure effectiveness [35]. Chemical methods comprise hyperosmotic/hyposmotic solutions, ionic detergents and non-ionic detergents; physical techniques are based on temperature (freeze–thaw cycles), hydrostatic pressure, mechanical agitation and sonication. Sterilisation techniques may be applied to provide a higher level of safety. There are significant advantages in combining decellularisation and sterilisation processes to ensure a clinically safe ECM, minimising the effect on its ultrastructure. Each method used for tissue decellularisation has to be standardised and validated. Then, standardisation and validation can be repeated in order to monitor the product obtained as well as to ensure maximum biological safety of decellularised tissues.

The common goal of all these methods is to obtain an acellular dermal matrix characterised by an intact fibrous and collagenous architecture, able to be repopulated by autologous cells of the patient after its engraftment. The absence of immune response and graft rejection in patients is ensured by removing the cellular components (fibroblasts and endothelial cells) as well as the donor DNA and hair remnants. From a functional point of view, these dermal matrices act as cell-free scaffolds able to permanently reconstruct and regenerate damaged and/or pathological skin tissue.

The main biological characteristics of an optimal dermal matrix are biocompatibility (the ability to take after engraftment and the absence of rejection/inflammatory reaction due to cytokine release), integrity of the matrix (the maintenance of integrity of elastic fibres and collagen physiologically identified in the tissue), sterility (absence of Gram+/− bacteria, fungi and bacterial endotoxins), malleability and suturability (handling; mechanical resistance with or without stitches) and storage options (the ability to be stored by different methods e.g. cryostorage in nitrogen vapours, storage at – 80 °C, freeze-drying, dehydration, room-temperature storage, storage in high-percentage glycerol).

See Chapter 8 for information on decellularisation of tissues as natural extracellular matrices.

Clinical indications of acellular dermal matrices are in the field of regenerative medicine and surgery and depend on wound thickness. Among them are:

- dermatology, plastic and reconstructive, general surgery and vascular surgery used for the treatment of acute (e.g. burns) and chronic (e.g. skin ulcers in various aetiology) skin wounds with extensive loss of substance: acellular dermal matrices with a thickness of 0.2-0.4 mm can be used in combination with a thin autologous split-skin graft to improve the scar quality of the wound; thicker acellular dermis is used for other indications such as reconstruction of the breast after mastectomy or hernia repair [36-46].
- orthopaedics for the repair of the rotator cuff of the shoulders as well as for the treatment of skin surgical wounds [47].
- maxillo-facial surgery, ENT surgery, dentistry for the sinus lift and implant dentistry for augmentation in gum reconstructions [48-49].

19.4.2. Processing facilities

In selecting an appropriate air-quality specification for skin processing, the criteria identified in Chapter 7 should be considered. Table 19.1 outlines the factors to be considered for skin processing.

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9584 **Table 19.1. Factors influencing the air-quality specification for processing of skin**

Criterion	*Skin-specific*
Risk of contamination of tissues or cells during processing	During processing, skin is necessarily exposed to the processing environment for extended periods.
Use of antimicrobials during processing	Soaking in antibiotics is the only anti-microbial step possible for cryopreserved skin, with maintained cell viability. To minimise the risks of particulate or microbial contamination of the product or materials being handled, it is indispensable to process tissues in cleanrooms (with air-quality standards as specified in Chapter 7). High concentrations of glycerol (85 %) used in the glycerolisation process have been shown to achieve long-term anti-microbial effect, though it cannot be considered a sterilising agent [50-51].
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of skin for microbiological analysis following antibiotic soaking is not extensive (random sampling). Sterility testing has significant statistical limitations, and can be used to detect only large-scale contamination. Final sterility testing may even be unreliable, especially if antibiotics remain on tissues [52]. Due to these limitations, aseptic methods must be used at all times.
Risk of transfer of contaminants at transplantation	Although skin is placed on the external surface of the body, it is mostly used for severely burned patients whose own skin barrier is no longer functional. These patients usually develop immuno-suppression by various mechanisms and, despite recent advances in therapy, they have a significant risk of death from infection.. As there is always a risk of transfer of contaminants by tissue transplantation, the demand for an aseptic method is obvious.

9585
9586 Taking the factors from Table 19.1 into consideration, skin grafts should be processed in optimal
9587 environments with air quality equivalent to Grade A in EU Good Manufacturing Practice (GMP)
9588 Guidelines, with an adequate background environment. For EU countries, the background must be at
9589 least Grade D but, given the risks associated with the use of skin grafts which are not sterilised or treated
9590 with equivalent microbial reduction methods, more stringent requirements are recommended.

9591 19.4.3. Sterilisation of skin allografts

9592 When tissue viability is not required or when skin tests positive for microbiological contaminants,
9593 it can be sterilised by gamma irradiation or electron beam. Ionising radiation (in relation to its dose) can
9594 cause structural changes in the irradiated skin allografts, especially in the epidermis [53]. Research has
9595 shown that a maximum dose of 25-kGy irradiation of deep-frozen skin in radio-protective solutions
9596 sterilises tissue without relevant histomorphological or physical alterations (such as pliability) compared
9597 with normal cryopreserved skin [11]. Sterilisation methods should be validated for the initial estimated
9598 level of bioburden prior to application of the sterilisation method to skin allografts.

9599 19.5. Quality control

9600 19.5.1. Microbiological control

9601 In addition to the standard microbiological controls described in Chapter 10, microbiological
9602 testing should be done before the start of processing and on post-processed samples of skin (without
9603 antibiotic) before the skin is approved for clinical use. These approaches are based on the
9604 microbiological test methods of *Ph. Eur.* and cover the minimum standards to control the
9605 microbiological safety of preparations of human tissues. Deviations from such standards should be
9606 justified and the suitability of the intended alternative test methods must be demonstrated and validated.
9607 Specimens of a representative sample of finished product (e.g. a predefined number of pieces of skin
9608 allografts that have undergone all stages of production) should be sent for microbiological testing to
9609 check for aerobic and anaerobic bacteria and fungi using appropriate culture media [27]. Acceptance
9610 criteria for microbial load and types of contaminant in processed tissues should be defined in advance
9611 and reported in written procedures.
9612 If a positive microbiology result is obtained at the initial stage or at an intermediate stage of
9613 processing, a risk-assessment analysis should be conducted to assess the suitability of the skin tissue

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9614 (and other tissues from the same donor), taking into account the micro-organism(s) detected. Basically,
9615 skin allografts may be accepted for clinical use, without sterilisation, when bacteriological and
9616 mycological assessment (refer to Chapter 10 for acceptable microbiological examination techniques)
9617 reveal only low bioburdens of inherent inhabitants of the residential skin flora. The surgeon shall be
9618 informed of the skin-graft bioburden before the use of the graft (see §19.9 for further information).
9619 Bioburden can be determined using quantitative cultures, but it is permitted to use more pragmatic
9620 validated approaches in which bacterial density is measured in terms of bacterial lawn confluency or the
9621 appearance of turbidity in periodically inspected liquid cultures. The presence of micro-organisms in
9622 finished product samples results in a definite rejection of the donor tissue if no validated sterilisation or
9623 decontamination method is applied (see Figure 19.2).
9624 For terminally sterilised skin, an equivalent analysis should be carried out, taking into
9625 consideration the capacity of the sterilisation process as demonstrated through validation.
9626 Specimens contaminated by (endo)spore-forming micro-organisms such as *Bacillus* and
9627 *Clostridium* spp. or any of the pathogens listed in Table 19.2, at any stage of the process (even if negative
9628 at the end of processing), should be discarded without corrective actions in order to remove potentially
9629 unsuitable tissue from the transplantation process. Table 19.2 is a suggested list of such micro-organisms
9630 that is non-exhaustive, can be updated and is subject to change, according to different geographical
9631 areas.

9632 **19.5.2. Skin allograft performance and quality issues**
9633 Viable donor skin is still considered the gold standard for the temporary covering of burns. In
9634 cryopreserved skin allografts, the viability of skin is often considered as an essential requisite and should
9635 be tested. Different methodologies are used to assess cell viability in skin grafts before and after thawing:
9636 • quantitative, e.g. tetrazolium salt assay (MTT), neutral red test (NRT), resazurin test, oxygen or
9637 lactate consumption assay [32-33];
9638 • and /or qualitative by histological staining, e.g. orcein, Masson, haematoxylin-eosin.

9639 Controversy exists in the literature data regarding the importance of cell viability in graft
9640 performance and quality. It is widely considered that viable skin allografts are superior to all other
9641 dressing materials, and the majority of physicians agree that higher viability is usually associated with
9642 better wound-bed preparation and graft take [32, 54-57]. Comparison of unprocessed, cryopreserved
9643 (viable) and glycerolised skin allografts by the use of animal studies (immuno-competent Balb/c mice)
9644 revealed a better performance (evaluated by histology) of unprocessed skin and, to a lesser degree, of
9645 deep-frozen (− 80 °C) and cryopreserved (in liquid nitrogen) skin [58]. These data demonstrated that
9646 graft performance of cryopreserved skin decreased with time.
9647 However, non-viable skin allografts can be successfully employed when viable cryopreserved
9648 skin allografts are not available or where cell viability is not required for wound treatment [4]. Literature
9649 studies [26] indicate that there is no evidence that viability of the graft influences healing outcomes.
9650 Thus, instead of viability, other aspects, such as structural integrity, clinical outcome and intrinsic anti-
9651 microbial safety of the preservation method and cost should be the primary criteria for the choice of
9652 preservation method to be used for allografts.

9653 19.6. Packaging and labelling

9654 The grafts are packaged in foil or polythene/polyethylene sterile containers and coded, packaged and
9655 labelled in accordance with the guidance in Chapter 14. All packages must be labelled with the name of
9656 the processing institution, a unique identifier or serial number linking the tissue to the donor, the expiry
9657 date, size and type of skin graft (e.g., cryopreserved, glycerolised).
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9659 ## 19.7. Storage

9660 Processed skin grafts are stored in various conditions depending on processing method. Glycerolised
9661 skin is usually stored in a bio-refrigerator at 2-8 °C. Lyophilised (freeze-dried) skin can be stored at
9662 room temperature. Cryopreserved skin can be stored in liquid or in the vapour phase of liquid nitrogen
9663 in a liquid nitrogen refrigerator. Viable (frozen/deep-frozen) skin allografts can also be stored in
9664 mechanical freezers, at −45/−60/−80 °C or in ultralow freezers (−130 °C), but at higher temperatures
9665 the storage time will be shorter (to maintain biological properties).

9666 ### 19.7.1. Expiry date

9667 In order to ensure the safety and quality of tissues and cells, the maximum shelf-life of tissue
9668 under each type of storage condition should be specified. The expiry or retest date should come from
9669 formal validation with stability studies, which should take into account, among other factors (e.g. expiry
9670 dates of reagents), possible deterioration of the required tissue and cell properties, integrity and stability
9671 of the packaging and labelling materials, according to the requirements of Chapter 7.
9672

9673

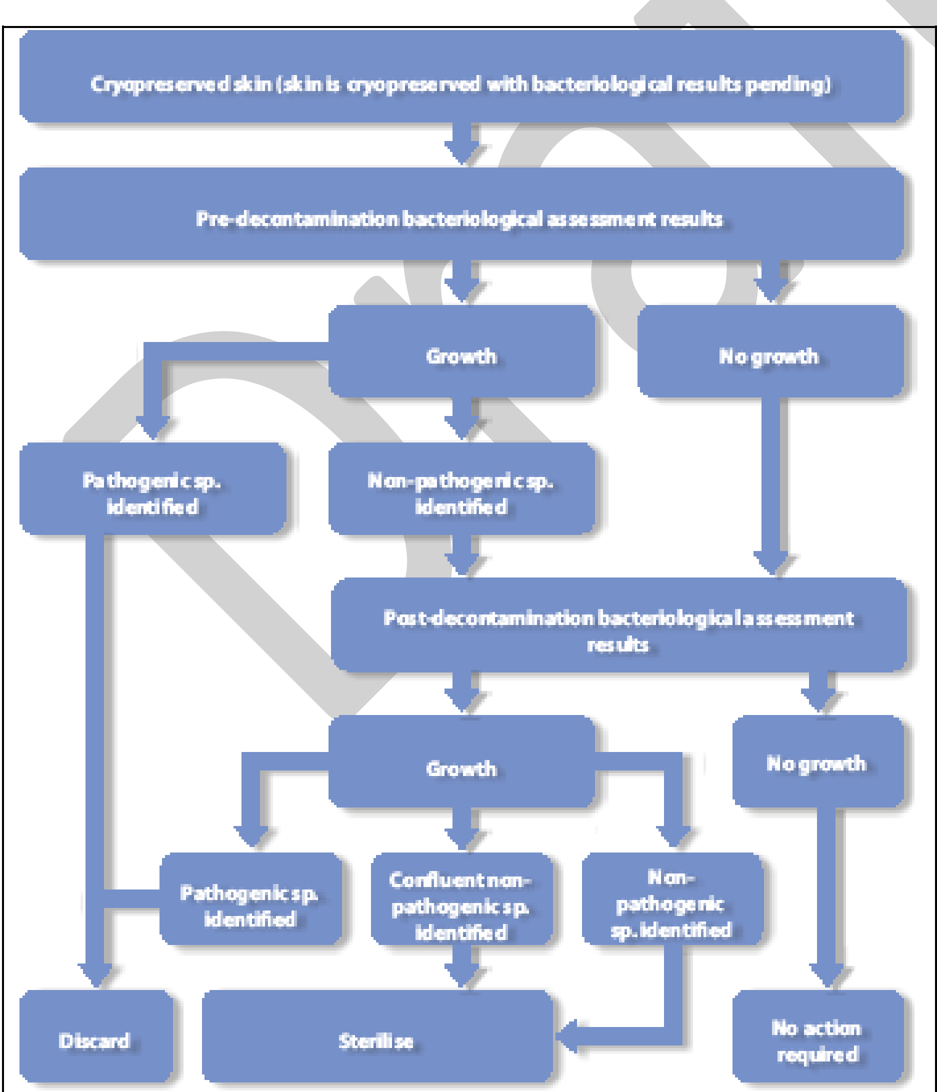

Figure 19.2. Algorithm for acceptance/rejection of skin after bacteriological assessment

Note: Pathogenic spp. identified during skin assessment should be understood as those defined in

Table 19.2. Contaminants that should result in tissue discard if detected at any stage of processing

Contaminants
Acinetobacter baumanni
Actinomyces
Bacillus anthracis
Bacteroides spp.
Burkholderia cepacia complex
Carbapenem-resistant *Enterobacteriaceae* (CRE)
Clostridium spp. (notably *C. perfringens* or *tetani*)
Corynebacterium diphtheriae
Erysipelothrix rhusiopathiae
Fusobacterium
Listeria monocytogenes
methicillin-resistant *Staphylococcus aureus* (MRSA)
Mucor spp.
Mycobacterium tuberculosis complex or *M. avium*
Neisseria meningitides or *gonorrhoeae*
Nocardia spp.
Pseudomonas aeruginosa
Salmonella typhi or *paratyphi*
Shigella spp.
Staphylococcus aureus
Stenotrophomonas maltophilia
Streptobacillus moniliformis
Streptomyces spp.
Vibrio cholerae
Yersinia pestis or *pseudotuberculosis*
Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

19.8. Skin allograft distribution

Skin allografts are considered life-saving therapeutic materials, so tissue establishments should have a written procedure for allocation of grafts based on clinical priority. Distribution of skin grafts for transplantation should be restricted to hospitals, tissue establishments, physicians, dentists or other qualified medical professionals, in compliance with national regulations and the WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation (Chapter 1).

19.9. Acceptance criteria and exceptional release

The acceptance criteria should be based on validated protocols and reported in the tissue establishment's written procedures. The release criteria and specifications of skin/dermal allografts should be defined, validated, documented and approved. There should be a defined procedure for exceptional release of non-standard skin/dermal allografts under a planned non-conformity system. The decision to allow such release should be documented clearly, and traceability should be ensured.

19.10. Biovigilance

Adverse events and reactions as well as serious adverse events and reactions shall be recorded, reported and investigated according to corresponding national regulations to the Health Authorities for tissues and cells.

19.10.1. Non-exhaustive list of reportable SARs

a. transmission of infective disease;
b. transmission of malignant disease;
c. allergic reaction (e.g. to antibiotics used for processing media);
d. engraftment failure/delayed engraftment (related to the tissue graft);
e. unexpected immunological reactions due to tissue;

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f. bleeding (wound bed preparation);
g. aborted procedure involving unnecessary exposure to risk (e.g. wrong tissue supplied or delayed transport, discovered after patient is anaesthetised and the surgical procedure has begun [52]. See also www.notifylibrary.org.

19.10.2.Non-exhaustive list of reportable SAEs

1. Procurement:
   a. procurement without consent;
2. Processing and labelling:
   b. use of non-sterile/expired materials for tissue processing;
   c. mistaken processing media (errors in media preparation);
   d. incorrect labelling;
3. Storage:
   e. storage at inadequate temperature (e.g. in case of equipment failure; unattended alarm);
4. Transport/distribution:
   f. loss of irreplaceable autologous tissues;
   g. delayed transport of cryopreserved skin (resulting in tissue discard);
   h. incorrect tissue type, i.e. a different type of tissue is supplied than was intended or requested;
5. Testing:
   i. bacterial/viral/fungal contamination of tissues distributed for transplantation;
   j. loss of cell viability in cryopreserved viable tissue.

The Notify Library (www.notifylibrary.org) includes some well-documented cases of adverse occurrences in skin transplantation. Examples include:

- Contaminated skin graft that caused serious infection of a burn wound with *Acinetobacter* (Record Number 428).
- A case involving distribution of cryopreserved skin without review of the results of bacterial tests. Several allograft recovery cultures showed virulent pathogens ordinarily not accepted for use that prompted recall of > 100 skin allografts, fortunately without any case of disease transmission (Record Number 128).
- Two cases describing incidents in which unsuitable skin grafts were released for clinical use. In one case, skin was torn upon thawing and implanting; in the second case, it was not measured appropriately, resulting in delay in patient treatment and graft loss in both cases (Record Numbers 126 and 127).

For further evaluated cases of adverse outcomes associated with skin banking, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

19.11. Developing applications

Wound healing is a major target in tissue-engineering research. In the past 40 years a large number of biological and synthetic skin/dermal substitutes have been produced. Products such as bioengineered skin equivalents and synthetic/biosynthetic materials integrated with cultured epidermal cells have been developed for this purpose, and most of them would be classified in the EU as advanced therapy medicinal products (ATMP) (Chapter 30). However, the ideal skin substitute has not yet been established and human skin allografts remain a gold standard in the therapy of major burns and skin loss treatment. Thus researchers in the field of tissue engineering are still working on the production of an ideal artificial skin able to act as a physiological skin.

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19.11.1.Epidermal cell suspensions

Epidermal cell suspensions (non-cultured autologous epidermal cellular grafting) have been used in the surgical management of vitiligo since 1992 when Gauthier and Surlève-Bazeille developed a non-cultured cellular grafting technique [59]. With this technique an epidermal cell suspension is used without cell expansion to treat larger areas (8- to 10-fold size of donor skin) on an outpatient basis with simple laboratory procedures. Epidermal cell suspensions can be useful in a variety of epidermal defects, involving both keratinocytes and melanocytes, and several approaches to delivering autologous keratinocytes/epidermal cells to restore epithelialisation have been developed [60, 61].

A recent technique based on aerosol spraying of non-cultured epidermal cells suspensions represents an efficacious and rapid way to obtain re-epithelialisation. To prepare the epidermal suspension, a cutaneous biopsy is trypsinised and epidermal cells are obtained by scraping of the dermal side of the epidermis. A suspension of autologous keratinocytes, melanocytes and fibroblasts can be delivered onto the wound bed by a spray apparatus. Therefore, epidermal cell sprays can be considered as aerosolised skin grafts that can potentially treat a variety of epidermal defects for burns and traumatic injuries, but also in scar reconstruction, donor-sites repair and in skin resurfacing techniques [60]. In consideration of the presence of melanocytes, this technique is also effective in pigmentation defects, including vitiligo and post-burn leukoderma.

Special commercial devices or prefabricated cellular preparation kits have also been developed to isolate and apply non-cultured epidermal cells, dermal cells or adipocytes to wounds in a one-step surgical procedure.

19.11.2.Epidermal 3D cell cultures

It was in 1975 that Rheinwald and Green [62] first described the serial cultivation of human keratinocytes in monolayer culture obtained from primary keratinocytes seeded onto lethally irradiated murine fibroblast feeder layers. Since then numerous advances have been made in the cultivation of human keratinocytes, in both two-dimensional monolayer and three-dimensional organotypic culture. Cultured epidermis was originally used to re-epithelialise severe burns, but, because of the presence of melanocytes, it was also used in vitiligo and other skin pigmentation disorders and to treat scars, ulcers and skin-graft donor sites.

Three-dimensional (3D) bioprinting, a flexible automated platform for the fabrication of complex living architectures, is a novel approach to the design and engineering of human organs and tissues [63]. A platform consists of eight independently controlled cell-dispensing channels that can precisely place cells, extracellular matrix (ECM), scaffold materials and growth factors in any user-defined 3D pattern. After the printing process, the skin tissue is cultured in media under submerged conditions to obtain a multi-layered cell and matrix structure in which human keratinocytes are grown on collagen matrices embedded with human fibroblasts.

All cell-culture methods are relevant in the field of tissue engineering and comply, when considered for clinical applications, with the ATMP regulations. Currently, the 3D bioprinter of skin is in the phase of being approved by different European regulatory authorities to guarantee that the skin that is produced is adequate for use in transplants on burn patients and those with other skin defects.

These tissues can be used to test pharmaceutical products, as well as cosmetics and consumer chemical products *in vitro* where current regulations require testing that does not use animals. See Part C for further information.

19.11.3.Skin composite grafts – nipple preservation

Practically any human tissue can be procured and banked for clinical use. Patients with loss of the nipple and areola from cancer, excision, trauma or congenital absence can undergo nipple-sparing mastectomy in specific cases after histological examination of the tissue surrounding the nipple and areola to eliminate the possibility of eventual cancer invasion. If a patient's nipple–areola complex (NAC) is available for grafting after mastectomy, it is the best material to use for reconstruction. It can

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be cryopreserved as a composite graft to be autografted for reconstruction of the breast after mastectomy. According to published literature and skin-bank protocols [64], a slow cooling procedure for cryopreservation is used by incubating the NAC in a cryoprotectant solution with 10 % DMSO.

The timing of transfer usually ranges from 6 months to 1 year after breast reconstruction. At the time of transfer, the cryopreserved NAC is thawed in 37 °C water and grafted on a projection made by a denuded dermal flap on the reconstructed breast.

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Related documents:

Appendix 16. Practical guidance for the evaluation of pigmented skin lesions and differential diagnosis of melanoma

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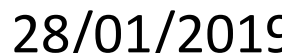

9928 # Chapter 20. Cardiovascular tissue

9929 ## 20.1. Introduction

9930 Cardiovascular tissues can be procured from deceased donors (after brain death or after circulatory
9931 death) and living donors (e.g. heart valves from a patient undergoing a heart transplant).
9932 The cardiovascular tissues most commonly procured are aortic valves, pulmonary valves and
9933 femoral arteries, but other cardiovascular tissues can be procured and processed, for example: ascending
9934 aorta, aortic arch, descending aorta, iliac arteries, aorto-iliac bifurcation, saphenous vein, vena cava with
9935 iliac veins, pulmonary conduits, patches dissected from pulmonary conduits and pericardium.
9936 Heart valves are used mainly in paediatric cardiac surgery for treatment of congenital
9937 malformations, such as tetralogy of Fallot, valve atresia, bicuspid or monocuspid aortic valve, or
9938 transposition of great arteries. The most important reason for clinical use of heart-valve allografts in
9939 these indications is the identical morphology of the allografts and the native valve of the patient, which
9940 enables easy reconstruction of the left/right ventricular outflow tracts (RVOT/LVOT). Another
9941 advantage is that recipients, who are mainly children and neonates, do not need long-term
9942 anticoagulation due to the non-thrombogenicity of human tissue, thereby avoiding the side-effects of
9943 anticoagulation therapy in these patients. Another important indication is the Ross operation, which is
9944 often used for young female patients of child-bearing age and for athletes. This entails replacement of
9945 the diseased aortic valve with the autologous pulmonary valve (autograft) and reconstruction of the
9946 patient's RVOT with a pulmonary (or aortic) allograft.
9947 The most common indication for the use of vascular (artery and vein) allografts is the infection
9948 of the prosthetic and/or native vascular tissue. Arteries may be used for peripheral re-vascularisation or
9949 reconstruction of the abdominal aorta. Iliac vessels are sometimes removed from deceased donors at the
9950 time of organ donation, and they can be used to support organ transplantation.
9951 In light of long-term follow-up studies, it has been identified that implanted cardiovascular
9952 allografts are the subject of premature deterioration, perhaps because of immune-related deterioration
9953 [1]. Therefore, methods for the reduction of immunogenicity in cardiovascular allografts have been the
9954 subject of much research during the past two decades. This has involved the development of new
9955 procedures to decellularise cardiovascular allografts. This may also facilitate recellularisation of the
9956 graft with cells from the recipient *in vitro* before implantation or *in vivo* after implantation.
9957 The following generic chapters (Part A) of this Guide all apply to cardiovascular tissue banking
9958 and must be read in conjunction with this chapter:
9959 a. Introduction (Chapter 1);
9960 b. Quality management, validation and risk management (Chapter 2);
9961 c. Recruitment of potential donors, identification and consent (Chapter 3);
9962 d. Donor evaluation (Chapter 4);
9963 e. Donor testing (Chapter 5);
9964 f. Procurement (Chapter 6);
9965 g. Premises (Chapter 7);
9966 h. Processing (Chapter 8);
9967 i. Storage and release (Chapter 9);
9968 j. Principles of microbiological testing (Chapter 10);
9969 k. Distribution and import/export (Chapter 11);
9970 l. Organisations responsible for human application (Chapter 12);
9971 m. Computerised systems (Chapter 13);
9972 n. Coding, labelling and packaging (Chapter 14);

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o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

This chapter defines the additional specific requirements for cardiovascular tissue.

20.2. Donor evaluation

20.2.1. Contraindications specific to cardiovascular tissue

The following exclusion criteria are specific to donation of cardiovascular tissue:

a. cardiac valvulopathy of the aortic and pulmonary valves, with moderate-to-severe stenosis or incompetence (the vessels can still be acceptable);
b. aortic dissection (detachment of the intima and adventitia);
c. direct (open) and massive traumas in the area of the body where the tissue is procured;
d. Marfan's syndrome and related diseases (e.g. Loeys–Dietz syndrome, Ehlers–Danios syndrome);
e. bacterial or fungal endocarditis.

Other conditions to be evaluated as part of the donor-selection process are:

a. myocardial dilatation and valve alteration such as dilated roots, wall alteration of aorta or pulmonary trunk;
b. untreated pneumonia in previous days due to highly pathogenic bacteria or fungi, such as *Staphylococcus aureus*, pneumococcus or *Candida*.
c. Previous surgical interventions on the tissue to be procured.

Donor age limits vary between centres, with validations being performed to extend limits, based on the specific evaluation of the quality of the tissue. In general, the quality of cardiovascular tissue deteriorates with increasing age, and maximum age limits are a simple way to apply donor-selection criteria. However, it must be considered that other lifestyle factors, such as diet, lifestyle and history of smoking also impact on the quality of cardiovascular tissue, though these are more difficult to assess quantitatively. Where clinical demand for particular types or sizes of cardiovascular graft is not being met, tissue establishments may choose to set higher age limits to increase the donor pool, in the knowledge that a significant proportion of the grafts donated may not be suitable for clinical application. Commonly applied age limits are shown in Table 20.1.

Table 20.1. Commonly applied age limits for cardiovascular donors

Arteries	male 17-45 years of age female 17-60 years of age
Aortic valves	32 weeks' gestation to 60 years of age
Pulmonary valves	32 weeks' gestation to 65 years of age

20.3. Procurement

20.3.1. Procurement team

The cardiovascular procurement teams should consist of at least two people. They should work under aseptic conditions, and be scrubbed, gowned in sterile clothing and wearing sterile gloves, face shields and protective masks.

20.3.2. *Post mortem* procurement time

It is recommended to procure cardiovascular tissue within 24 h after death, but only if the body has been cooled or refrigerated within 6 h of death. If the body was not refrigerated, then it is possible

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10010 to procure tissue in the first 12 h after death. It may be possible to extend procurement times up to 48 h
10011 after death if subsequent processing has been validated to guarantee quality and microbiological safety;
10012 in these cases the blood samples for serological testing should be taken within 24 h after death (to avoid
10013 extensive haemolysis). See Chapter 5 for details of sample collection.

10014 **20.3.3. Procurement procedure**

10015 Procurement should be carried out in an environment that is as clean and controlled as possible,
10016 ideally in the operating theatre, or in a suitable environment supported by risk assessment. For heart-
10017 valve procurement it is important to procure the ascending aorta and the pulmonary trunk with
10018 bifurcation (wherever possible) together with the heart. All efforts should be made to procure as much
10019 length of pulmonary artery distal to the pulmonary bifurcation as is practicable.

10020 For vessel donors, the maximum possible length of the recovered vessel should be maintained,
10021 avoiding iatrogenic lesions during manipulation, and collateral branches should be cut 2-3 mm from the
10022 arterial wall to allow the surgeon to ligate or suture them during the surgical procedure to avoid
10023 unnecessary bleeding in the patient.

10024 **20.3.4. Tissue transportation to the tissue establishment**

10025 Common practice is to place procured tissues in a crystalloid transport solution (e.g. physiological
10026 saline, Ringer solution, Hanks balanced salt solution) with the possible addition of nutritional/osmotic
10027 elements (e.g. albumin) or antibiotic cocktail, and package them in at least two sterile packaging layers
10028 after procurement. The transport solution should also be buffered to ensure a physiological pH is
10029 maintained during transport.

10030 For donors of organs, valves and vessels, it is convenient to package the heart and the vascular
10031 segments in different containers to avoid potential contamination transmission.

10032 This package should then be placed in another container that ensures an ambient temperature of
10033 2-8 °C during transport, and protects the recovered tissues.

10034 **20.3.5. Procurement documentation**

10035 The organisation responsible for procurement must produce a procurement report to be given to
10036 the tissue establishment. In addition to the generic requirements defined in Chapter 6, this report must
10037 contain a description and identification of the recovered material (heart, arteries, veins, valves, etc.).

10038 20.4. Processing and storage

10039 Procured cardiovascular tissues can be processed to facilitate longer storage periods and to reduce
10040 microbial contamination. To ensure tissue quality, it is essential that the time between cardiac arrest and
10041 cryopreservation be as short as possible. Time from procurement of the heart to dissection and
10042 disinfection should not exceed 24 h. The total ischaemia time (cardiac arrest to cryopreservation and
10043 storage) should not exceed 72 hours.

10044 **20.4.1. Cardiovascular tissue-processing methods**

10045 Processing of cardiovascular tissues includes dissection and evaluation of morphology and
10046 minimum functional requirements, incubation with antibiotics and, in some cases, anti-mycotics,
10047 cryopreservation and storage. The duration and temperature of antibiotic treatment and the composition
10048 of antibiotic cocktails should be defined by each tissue establishment, with prior evaluation of the initial
10049 tissue bioburden (i.e. before the tissue comes into contact with an antibiotic solution) and following a
10050 validation of the effectiveness of the cocktail against the most common microbes likely to contaminate
10051 the tissues. Tissue establishments should establish a clear policy stating how pre- and post-
10052 decontamination microbiology results will be used to determine whether the grafts are suitable for
10053 clinical use.

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10054 The methods used must be in accordance with current state-of-the-art and validated procedures
10055 (see Chapter 2). Different tissue establishments apply specific preparation processes according to their
10056 own standard operating procedures (SOPs) and in accordance with relevant local authorisations.
10057 As cardiovascular tissue is not visible for inspection once it has been cryopreserved it is
10058 recommended that the anatomical appearance, quality and other attributes noted during processing are
10059 documented to assist with the allocation of a suitable allograft for patient need. Appendix 27 provides
10060 an example of an evaluation form.
10061 The annular diameter of valves and vessels should be measured using calibrated obturators. The
10062 length of the vessels should be recorded, as should the approximate position and size of any branching
10063 vessels. It is recommended that grafts be measured immediately prior to preservation, as measurements
10064 may alter following procurement [2].

10065 **20.4.2. Decellularisation of cardiovascular tissues**

10066 Heart valves and large vessels can be decellularised employing different methods to eliminate
10067 cellular components. Decellularisation protocols can employ physical methods (freezing, sonication),
10068 chemical methods (hyperosmotic solutions, ionic detergents, non-ionic detergents) and enzymatic
10069 methods (trypsin, endonucleases). The most robust and effective decellularisation protocols include a
10070 combination of the three methods (see Appendix 32) [3, 4]. Quality control should guarantee
10071 maintenance of the structure and the biomechanical properties of native valves and vessels, as well as
10072 demonstrating *in vivo* function. It must be shown that residual quantities of any reagents used during the
10073 decellularisation process that are still present in the tissue do not provoke cytotoxic responses either *in*
10074 *vitro* or *in vivo*. Decellularisation protocols (especially enzymatic methods) should take into account that
10075 degraded collagen might have repercussions for in vivo re-endothelialisation of decellularised tissues.

10076 **20.4.3. Processing facilities**

10077 In selecting an appropriate air-quality specification for processing cardiovascular tissue, the
10078 criteria identified and explained in Chapter 7 should be considered alongside the factors outlined in
10079 Table 20.2.
10080 It is vital that the processing of cardiovascular allografts takes place in a microbiologically and
10081 physically controlled environment with temperature control, ventilation and air filtration, and with
10082 validated cleaning and disinfection. Taking the factors from Table 20.2 into consideration,
10083 cardiovascular tissue should be processed in optimal environments with air quality equivalent to Grade
10084 A in EU Good Manufacturing Practice (GMP) Guidelines, with an adequate background environment.
10085 For EU countries, the background must be at least Grade D but, given the risks associated with the
10086 processing, testing and implantation of cardiovascular tissues, it is recommended that as a minimum, a
10087 Grade B or C background environment (EU GMP) be provided.
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10090 **Table 20.2. Factors influencing the air-quality specification for processing of cardiovascular tissue**

Criterion	*Cardiovascular tissue-specific*
Risk of contamination of tissues or cells during processing	During processing, heart valves and vessels are exposed to the processing environment for extended periods during dissection, sizing and evaluation of their characteristics.
Effectiveness of decontamination process	Heart valves and vessels are exposed to antibiotics, and in some cases, antimycotics, with a typical decontamination period of 24 h. It is important to validate the effectiveness of the antibiotic cocktail and to list the micro-organisms that can be accepted pre-incubation because this method is not very effective compared to more robust methods that can be applied to other tissues [5].
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of a piece of myocardium or a discarded vessel for microbiological analysis does not ensure a representative sample for analysis. Storage media or solutions used to rinse the tissue should also be sampled to make this evaluation more effective. Post-decontamination microbiology testing may not give a true reflection of the microbial status of the graft due to carry-over of antibiotics used in the decontamination process.
Risk of transfer of contaminants at transplantation	Cardiovascular tissue is vascularised and can support and transmit microbiological contaminants. Cardiovascular tissue is used in open surgery in well-vascularised areas and frequently to replace infected tissue (endocarditis). If it is contaminated, the risk of serious infection is considerable.

10091

10092 ## 20.5. Cryopreservation and storage

10093 Cardiovascular tissues can be cryopreserved by using a controlled-rate freezer and following a validated
10094 protocol. During the cryopreservation process, the parameters of the freezing cycle must be recorded,
10095 as well as any inconsistencies that might have occurred during the operation. After cryopreservation, the
10096 frozen tissues can be transferred to a temperature-monitored vessel (either a liquid nitrogen tank or
10097 mechanical freezer) and stored at < -140 °C. Cardiovascular tissue can be stored at < -140 °C for a
10098 storage period supported by validation data or a documented rationale based on maintenance of the
10099 critical properties of the graft.

10100 ## 20.6. Quality control

10101 It is recommended that the quality-control tests on vascular grafts should consider the following
10102 minimum quality criteria:
10103 a. integrity of the vascular walls;
10104 b. minimal calcification, atheroma and fibrosis;
10105 c. anatomical suitability – do not accept if aneurism or stenosis present.

10106 Quality-control tests for heart valves should consider the following minimum quality criteria:
10107 a. Functional competence. It should be noted that fenestrations within the margins of the lunulae are
10108 very often not a pathological finding. Provided the coaptation of the graft is ensured by adequate
10109 sizing, marginal fenestrations should not induce valve regurgitation either in the short or long
10110 term. Large fenestrations, particularly when they are in opposing cusps, should constitute a
10111 rejection criterion. Additionally, low-positioned fenestrations in the leaflets with moderate to
10112 severe leak should constitute a rejection criterion.
10113 b. Good morphology (no fissures, no congenital defects, no/minimal calcification, or no other
10114 significant anatomical abnormality). Only small calcifications in the distal wall of the aorta or
10115 around the coronary ostia, where they are most likely not to interfere with graft functioning, can
10116 be accepted, although information on their size and location must be clearly reported to the clinical
10117 user.

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c. Anatomical suitability (i.e. accurate length of conduit and diameter of annulus). Special attention should be paid to achieving an accurate measurement of the diameter of the annulus to avoid overstretching; this is particularly critical for the pulmonary valve.
d. Intact structure of the tissue matrix.

Cardiovascular allografts must be microbiologically sampled and cultured for aerobic and anaerobic bacteria, as well as fungi and yeasts, according to *European Pharmacopoeia* criteria (see Chapter 10), before antibiotic and, where relevant, antimycotic incubation.

Microbiological analyses should be carried out on:

a. the transport medium at the beginning of the processing procedure;
b. the sub-valvular (aortic and pulmonary) myocardial tissue and vessels before antibiotic incubation;
c. a final sample of each graft after antibiotic/antimycotic incubation and rinsing, and a sample of the cryoprotectant solution.

The result of the microbiological control must be negative. If a positive microbiology result is obtained, depending on the micro-organism found, a risk-assessment analysis should be done to assess the suitability of the other valve (and the rest of the tissues) obtained from the same donor. Table 20.3 lists some micro-organisms that, if detected in any culture of cardiovascular tissue (even if detected just before decontamination) require the tissue to be designated as unsuitable for clinical use. Hence, for example, detection of *Enterococcus* spp. in a pre-antibiotic sample of aortic myocardium with a negative result in all other samples (e.g. transport medium, post-decontamination aortic sample, before final packaging) should result in rejection of all valves from this donor, and a risk assessment should be done for the remainder of the tissues.

It should be noted that Table 20.3 is a suggested, non-exhaustive list, and individual tissue establishments may have a different list of micro-organisms that result in tissue discard.

Table 20.3. Contaminants that should result in tissue discard if detected at any stage of processing

Aspergillus spp.
Candida spp.
Clostridium spp. (notably *C. perfringens* or *C. tetani)*
Enterococcus spp.
Flavobacterium meningiosepticum
Klebsiella rhinoscleromatis
Listeria monocytogenes
methicillin-resistant *Staphylococcus aureus* (MRSA)
Mucor spp.
Mycobacterium spp.
Neisseria gonorrhoeae
Nocardia spp.
Penicillium spp.
Pseudomonas aeruginosa or *P. pseudomallei*
Salmonella spp.
Shigella spp.
Streptococcus pyogenes (Group A)
Other yeasts and fungi
Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

20.7. Cardiovascular allograft distribution

Transportation of cardiovascular tissues can be carried out using dry-shipping containers (vapour phase nitrogen < −140 °C). This allows re-storage of the tissues in the liquid or vapour phase of nitrogen without affecting the expiry date. If the tissue is to be stored at −80 °C, for example following issue to

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an end user, expiry date must be reduced to a time period supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

If dry ice is used for transportation of the vascular allograft, the tissue should not be returned to liquid or vapour phase nitrogen tanks unless validated.

Transport temperatures above −60 °C for cryopreserved cardiovascular tissues are to be strictly avoided to ensure the stability of the product and maximum safety for the recipient. The receiving tissue establishment must ensure that all packaging and distribution processes have been carried out under controlled conditions.

20.8. Cardiovascular tissue thawing

Thawing, removal of the cryoprotective medium (dilution) and re-establishment of the isotonic state of the cardiovascular allograft are of critical importance in order to guarantee the integrity of the cryopreserved tissue. The record that accompanies the cryopreserved tissue must contain the detailed protocol to be used for thawing, dilution and tissue reconstitution, together with a comprehensive list of the materials required. Where cryopreserved grafts are thawed directly from vapour or liquid nitrogen, for example if they have been transported in a dry-shipper, care must be taken to ensure that rapid thawing does not cause thermal shock which can result in microcracks in the grafts. Once cardiovascular tissues have been thawed, they cannot be re-frozen and should be implanted as soon as possible. A maximum period between thawing and transplantation should be defined, based on validation data or a documented rationale.

20.9. Examples of serious adverse reactions/events

The Notify Library includes some well-documented cases of adverse occurrences in the transplantation of cardiovascular tissue. Examples include:

- Donor-to-recipient transmission of hepatitis C virus (HCV) by transplantation of a saphenous vein after confirmation of transmission to a tendon recipient from the same donor. Imputability was confirmed by detection of identical HCV genotype 1a and phylogenetic nucleic acid arrangement (Record Number 564).
- Transmission of hepatitis B virus by aortic valve allograft resulting in asymptomatic seroconversion in the recipient (Record Number 424).
- Serious adverse events such as an incorrectly sized heart-valve package opened by mistake (Record Number 122) and the heart valve determined to be unusable due to excess tissue attachments (Record Number 123), both resulting in delay in treatment and graft loss [6].

For further evaluated cases of adverse outcomes associated with banking of cardiovascular tissue, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

Typical serious adverse reactions or events that may occur with cardiovascular grafts and that should be reported include:

- post-implantation infection ;
- any factors suggesting rapid degeneration/deterioration/failure of the graft, e.g. regurgitation with heart valves, or stenosis/claudication with vessels.

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10187 ## 20.10. Developing technologies and applications

10188 ### 20.10.1. Veins

10189 In addition to the long-established vascular allografts such as pulmonary valves, aortic valves and
10190 femoral arteries, over recent years there have been several publications on the use of veins, particularly
10191 saphenous veins [7, 8].
10192 The suggested clinical indications for saphenous veins include:
10193 • peripheral vascular disease,
10194 • coronary artery bypass grafting (CABG),
10195 • patients with infected fields or at risk of infection,
10196 • arteriovenous access.

10197 The benefits for saphenous veins are broadly similar to other vascular allografts:
10198 • morphologically similar to the native tissue,
10199 • resistance to infection,
10200 • alternative to autologous veins.

10201 The processing and storage protocols for saphenous veins are the same as those employed for
10202 other vascular allografts in that they have venous branches ligated, and they are antibiotic-treated and
10203 cryopreserved.
10204 Other clinical indications for veins are currently being explored, as are other processing
10205 techniques such as decellularised veins.

10206 ## 20.11. References

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10224 **Related document:**

10225 **Appendix 27. Sample form for the evaluation of heart valves.**
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Chapter 21. Musculoskeletal tissue

21.1. Introduction

Human bone and soft-tissue grafts are being used increasingly in surgery as valuable materials to rebuild and replace musculoskeletal structures. Bone is the most commonly banked and transplanted tissue.

Fresh autologous bone is considered to be the ‘gold standard’ in bone-grafting material because it combines all the properties required in a bonegraft material: osteoinduction – i.e. bone morphogenetic proteins (BMP) and other growth factors – with osteogenesis (osteoprogenitor cells) and osteoconduction (scaffold). However, use of autografts is limited by the amount that can be procured and the risk of donor-site morbidity; so, in most cases, allografts are used.

Allografting of bone and musculoskeletal soft tissues can in most cases allow adequate and predictable restoration and functionality, including mechanical properties, analogous to the original healthy tissue at the recipient site. In addition, bone allografts offer the benefit of osteoconductive properties or even, depending on the processing applied, different degrees of osteoinduction through growth factors originally present and preserved in the graft [1, 2].

Musculoskeletal tissues can be procured from donors after brain death, donors after circulatory death and living donors (e.g. in the case of a patient undergoing hip or knee prosthesis surgery), and such tissues include bones, ligaments, tendons, cartilage and other soft tissues (e.g. fascia lata). The current indications for the transplantation of musculoskeletal tissues are, but are not limited to, tumour surgery, prosthesis replacement, filling where there is bone loss, fractures, malunion, bone fusion (spine and limbs), and ligament and meniscus replacement.

Allogeneic bone can be processed in different ways, depending on clinician needs and preferences. The processing methods include cutting or grinding into morcellised and cancellous chips, corticocancellous rings and wedges, and cortical grafts such as struts and cylinders. Bone grafts can be frozen or freeze-dried, and in some cases bone grafts are demineralised to enhance the osteoinductive properties; the result is demineralised bone matrix (DBM). Large osteochondral grafts and whole-bone segments are also provided, matched to the requirements of the recipient site [3].

This chapter defines the specific requirements for musculoskeletal tissue donation, donor evaluation, processing and preservation procedures that meet adequate quality and safety standards.

The future of musculoskeletal tissue banking is focused on the following areas:

a. developing new preservation methods to maintain the biological properties of the grafts;
b. developing new procedures such as decellularisation or specific cell seeding to improve graft incorporation in recipients;
c. improving the safety of grafts.

The following generic chapters (Part A) of this Guide all apply to musculoskeletal tissue banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, risk management and validation (Chapter 2);
c. Recruitment of potential donors, identification and consent to donate (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);
h. Processing (Chapter 8);
i. Storage and release (Chapter 9);

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10271 j. Principles of microbiological testing (Chapter 10);
10272 k. Distribution and import/export (Chapter 11);
10273 l. Organisations responsible for human application (Chapter 12);
10274 m. Computerised systems (Chapter 13);
10275 n. Coding, labelling, and packaging (Chapter 14);
10276 o. Traceability (Chapter 15);
10277 p. Biovigilance (Chapter 16).

10278 This chapter defines the additional specific requirements for musculoskeletal tissue.

10279 21.2. Donor evaluation

10280 General exclusion criteria are described in Chapter 4. Musculoskeletal tissue-specific criteria are
10281 described below.

10282 21.2.1. Musculoskeletal tissue: specific exclusion criteria

10283 In addition to the general exclusion criteria described in Chapter 4, screening of donors of
10284 musculoskeletal tissue should be conducted for:
10285 a. diffuse connective-tissue disease;
10286 b. metabolic bone diseases (severe osteoporosis, osteopetrosis, Paget disease, etc.);
10287 c. corticoid treatment (medical director should evaluate donor suitability depending on corticoid
10288 dose and treatment duration);
10289 d. evidence that the donor has ingested, or been otherwise exposed to toxic substances that could be
10290 transmitted in donated material in dosages that could endanger the health of recipients (e.g.
10291 cyanide or heavy metals such as mercury or gold);
10292 e. local bacterial, viral, parasitic or mycotic infection;
10293 f. radiation exposure at the location of the tissue to be donated (chest X-ray could be accepted);
10294 g. evidence of trauma (e.g. open fracture) at the procurement site, or presence of joint deformities
10295 (evaluate the possibility of contraindication for osteochondral, structural bone and/or cartilage);
10296 h. iatrogenic or degenerative tears or lesions detected during procurement of cartilage, menisci,
10297 tendons and osteoarticular grafts;
10298 i. poor nutritional status, which may occur in, among others, donors with a history of alcoholism
10299 and can lead to reduced bone quality.

10300 Donor age limits differ for different types of musculoskeletal tissue. These limits may be revised,
10301 based on performance of a validation study. Some countries have national guidelines or requirements
10302 but, in their absence, the following age limits, for male or female donors, are recommended:
10303 a. for bone, the minimum age for both sexes is 6 years. No upper limit is applied unless the bone is
10304 intended to be used for structural support, in which case younger donors (age 15-55 years) are
10305 preferred;
10306 b. for osteoarticular grafts, cartilage and menisci, the age range is 15-45 years;
10307 c. for tendons and fascia lata, the age range is 15-65 years, although the upper limit can be extended
10308 after a biomechanical validation study

10309 21.3. Procurement

10310 General principles of procurement are described in Chapter 6.

10311 21.3.1 Procurement team in deceased donors

10312 It is recommended that the musculoskeletal procurement team for deceased donation should be
10313 composed of at least two (but preferably three) people. The number of people involved in procurement

should be determined in advance depending on the amount of donated tissues per procurement procedure. To minimise the risk of contamination during procurement it is recommended to limit the maximum number of team members [4]. Procurement team members should work under aseptic or clean conditions (clean conditions could be accepted if a validated sterilisation procedure is included in the manufacturing process) and, after hand disinfection, they should be gowned in sterile clothing and wearing sterile gloves, face shields, glasses and protective masks.

Staff must have the experience, education and training necessary to procure tissues, including significant anatomical knowledge to accurately obtain not only the regular tissues procured (femur, patellar ligaments, etc.), but also specially requested materials (e.g. whole elbow).

It is important to define the functions of the individual members of the team for the different procurement processes (e.g. donor preparation, draping, procurement, microbiological sampling, packaging, reconstruction) and also to define the role of the team leader or person responsible for procurement.

21.3.2 Procurement procedures

The methods of tissue procurement may be similar to those used by orthopaedic surgeons in the operating room or may use wider skin incisions, applying strict aseptic techniques.

The steps for musculoskeletal procurement are:

a. Donor preparation: it includes washing, shaving and pre-operative disinfection of skin to reduce transient and resident microbial flora;
b. Donor draping: a local sterile field using disposable sterile drapes must be established before procurement to effectively reduce risk of microbial contamination;
c. Tissue procurement: all tissues must be procured using an aseptic technique. It is recommended that some rules are established to decrease the risk of cross-contamination, for instance:
   i. Personnel located at one side of the donor should not change to the other side until they have finished procuring all tissues from their own side. Surgical instruments should not be shared between personnel;
   ii. The packaging area should be independent and separate from procurement areas; but in an area with same environmental conditions;
   iii. It is recommended that a fixed procurement sequence is established, from ‘cleanest’ (e.g. lower limbs) to ‘dirtiest’ areas (e.g. abdominal cavity);
   iv. It is recommended to change gloves and surgical blade after procuring the tissues from one area (e.g. left leg).
d. Microbiological control: it is recommended to perform a microbiological control on each procured piece (swabbing, biopsy). Such controls can be avoided only when a validated sterilisation method is further applied during processing. Sampling methods should be consulted with a microbiological laboratory and defined in SOPs;
e. Tissue packaging: procured tissue must be inspected and identified appropriately before packaging and labelling to avoid mix-ups (see Chapter 14). Musculoskeletal tissue shall be packaged in a manner that minimises contamination risk, using a validated packaging system, to assure its isolation from the external environment;
f. Donor reconstruction (see §21.3.3);
g. Procurement documentation (see §21.3.5).

The musculoskeletal tissues most frequently procured from deceased donors are:

- long bones (femur, tibia, fibula, humerus, radius, ulna, rib);
- irregular bones (iliac crest, hemipelvis, vertebrae, skull, sternum, clavicle, scapula, mandible);
- soft tissues:
    - tendons: patellar, Achilles, anterior and posterior tibialis, peroneus longus, gracilis, semitendinosus;

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10362 o cartilage: meniscus, acetabular labrum, costal cartilage;
10363 o fascia lata;
10364 o *dura mater* (note: CJD/vCJD risks)

10365 Musculoskeletal tissues can also be procured from living donors:
10366 • Allograft
10367 o Patients having a hip-replacement procedure can donate the femoral head that is being
10368 replaced by the prosthesis, and in some cases bone removed in knee replacement is
10369 also banked. This can be frozen or further processed and provided to other patients as
10370 a bone graft.
10371 • Autograft
10372 o Cranial flaps removed during neurosurgical procedures where there is brain oedema.
10373 The tissue is stored and replaced in the same patient once brain swelling has
10374 diminished;
10375 o Cartilage can be used for producing autologous chondrocyte cultures for application in
10376 the same patient (see Chapter 32).

10377 **21.3.3 Reconstruction of the deceased donor's body**
10378 Once tissues have been procured from a deceased donor, the body must be reconstructed to
10379 maintain its original anatomical appearance.
10380 For aesthetic reasons and with a view to a respectful reconstruction of the donor, a wooden or
10381 other replica (note: funeral requirements) bone approximating the size of the donated bone may be used
10382 to replace the procured bone. The subcutaneous tissue and skin should be sutured. The use of sutures
10383 and other materials suitable for cremation should be considered.

10384 **21.3.4 Temporary storage and transportation to the tissue establishment**
10385 Once procured, if maintenance of cell viability is not crucial, musculoskeletal tissues should be
10386 kept at ≤−15 °C until they are transported to the tissue establishment. If transport occurs immediately
10387 after procurement, tissues must be refrigerated preferably not longer than 12 hours.
10388 If tissues are obtained to be preserved unprocessed (e.g. osteochondral grafts) or during temporary
10389 storage before processing, they can be placed in a transport solution buffered at a physiological pH (e.g.
10390 Ringer's lactate solution, Hank's balanced salt solution) with the possible addition of nutritional/osmotic
10391 elements (e.g. albumin), antibiotic cocktail or culture medium, and packaged in at least two sterilised
10392 packaging layers after procurement. This package should then be placed in another container that ensures
10393 a temperature of 2-10 °C.
10394 Temperature during temporary storage and transport as well as duration of temporary storage and
10395 transport should be validated for the related tissue to ensure protection of the procured tissues'
10396 properties.
10397 Temporary storage must provide clearly separate and distinguishable areas for tissues and cells
10398 that remain in quarantine. To prevent mix-ups or cross-contamination, physically separate areas, storage
10399 devices or secured segregation within a storage device/unit (i.e. refrigerator, freezer) must be allocated
10400 and prominently labelled (including at least the minimum required information – see Chapter 14).
10401 Temporary storage areas or units for tissues and cells must be monitored (and alarmed, if necessary) and
10402 checked to ensure expected environmental requirements are being met.

10403 **21.3.5 Procurement documentation**
10404 The organisation responsible for procurement must gather all relevant information associated with
10405 procurement procedures and produce a report to be given to the tissue establishment. In addition to the
10406 generic requirements defined in Chapter 6, this report must contain:

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a. description and identification of the procured material (specifying all procured tissues);
b. any relevant morphological detail of procured tissues;
c. presence of lesions, including those produced during procurement;
d. non-procured standard tissues and its reason.

21.4. Processing methods

21.4.1 Processing facilities

In selecting an appropriate air-quality specification for musculoskeletal tissue processing, the criteria identified in Chapters 7-8 should be considered.

All stages of tissue processing should take place within a controlled environment. Although classified clean rooms are often not formally required for initial processing steps when validated sterilisation and virus-inactivation processes are applied subsequently, it is nevertheless necessary to control media quality (especially air and water) and to work with appropriately disinfected or sterilised equipment.

For terminally sterilised grafts, at a minimum, the packaging step after cleaning and/or disinfection, but before sterilisation, should be conducted in a qualified clean room (see EU Good Manufacturing Practices classification). The official requirements vary between jurisdictions, but EU GMP Grade C is usually specified (WHO TRS 823, 1992).

For non-terminally sterilised grafts, the requirements for the processing environment depend upon whether the national authority mandates conformity with the EU GMP guidelines, or whether less stringent guidelines – e.g. the GTP (good tissue practice) guidelines – are applicable.

All jurisdictions and guidelines require that aseptic processing (post-sterilisation manipulations and transfers of 'open product') be conducted under EU GMP Grade A surrounded by a Grade B environment (so-called A-in-B), or it should be located within a validated isolator. For other tissue grafts, e.g. bone destined for terminal sterilisation, a surrounding Grade C or even D environment (i.e. A-in-D) may be acceptable. Some factors which could influence the air quality for processing of musculoskeletal tissue are given in Table 21.1.

Table 21.1. Factors influencing the air-quality specification for processing of musculoskeletal tissue

Criterion	*Musculoskeletal tissue-specific*
Risk of contamination of tissues or cells during processing	During processing (including cutting, shaping, cleaning, grinding, etc.), musculoskeletal tissue is necessarily exposed to the processing environment for extended periods. Environmental conditions are not as critical during freeze-drying if the tissues are packaged in a validated closed system during the freeze-drying procedure.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling can be done by swabbing, immersion of a tissue biopsy in culture medium after processing or by filtering and culturing washing solutions. Different sampling methods can be combined in order to detect the possibility of contamination. For bone that is processed to small pieces or ground, representative samples can be taken for culturing. For sterilised bone, sampling is not an issue as the process is validated to achieve a certain inactivation level for micro-organisms.
Risk of transfer of contaminants at transplantation	Bone marrow, lipids and blood components placed inside grafts act as a reservoir of micro-organisms. Decontamination methods act by removing these components from musculoskeletal tissues to decrease the risk of transmission of viral and bacterial agents. Musculoskeletal tissue is used in open and well-vascularised surgeries, sometimes linked to replacement of a prosthesis, where a significant risk of infection exists.

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21.4.2 Cleansing (physical preparation/defatting)

The methods of musculoskeletal tissue processing vary between individual tissue establishments. Allogeneic and autologous bone allografts from living donors can be processed in the same manner as tissues from deceased donors.

The initial processing of bone and other musculoskeletal tissue generally involves mechanical steps that remove extraneous tissue. In the case of bone, residual muscle tissue and periosteum are resected, and cartilage may be debrided. Thereafter, initial pieces of musculoskeletal tissue are cut and possibly drilled and/or planed and/or shaped and subjected to additional physical or chemical downstream processing.

Residual bone marrow, lipids and blood components in and/or on tissue can have a negative effect on subsequent processing and final graft quality. Such residues may increase bioburden and/or have a negative effect on sterilisation processes and might be a cause of immunogenic reactions or delayed incorporation in graft recipients. They may also contain infectious agents derived from the donor. Such residual tissues should therefore be removed. This is generally achieved with washing processes that may combine physical and chemical components including debridement, purging (with water, saline or organic solvent solutions), ultrasound and treatment with supercritical carbon dioxide [5, 6]. High-concentrated alcohol or comparable solutions could improve the defatting process [7]. Where appropriate, delipidation processes should reliably reduce the intrinsic cellular bioburden to a level that can be eliminated/inactivated by subsequent processes [8-10].

All processes should be validated. The tissue establishment should determine what properties of the allograft are essential for safe and effective clinical application, select appropriate tests to evaluate these properties and design a validation plan accordingly. The tissue establishment may select to validate individual processes for individual graft types, or may choose a worst-case validation plan to cover all graft types. The latter should be based on the results of a risk assessment.

The types of graft that can be obtained include (but are not limited to):

- cancellous and corticocancellous chips or cubes, e.g. obtained from epiphyses of the long bones, vertebral bodies, or os ilium;
- cortical chips e.g. obtained from diaphysis of long bone;
- bone blocks (cancellous, corticocancellous), e.g. obtained from epiphyses of the long bones or vertebral bodies;
- bone wedges, e.g. obtained from epiphyses, os ilium, calcaneus or talus;
- the whole bones;
- structural bone segments (whole or halved diaphyses, rings, struts or condyles);
- patellar or Achilles tendons with bone blocks;
- tendons without bone blocks;
- menisci, either whole (with or without bone blocks) or sections;
- fascia lata patches;
- costal cartilage segments.

Pooling of musculoskeletal tissue from multiple donors during processing is not recommended (see Chapter 8). However, in some countries pooling is permitted for some grafts (e.g. cancellous tissue). In such cases risk assessment is mandatory, taking into consideration the increased risks for the patient due to increased donor exposure and balancing benefits of the treatment.

Bone grafts should support bone healing. Appropriately processed bone grafts will provide ‘osteoconduction’ (i.e. they act as a scaffold and ‘guide rail’ for osteoclasts and osteoblasts) and therefore promote the incorporation of the graft and its remodelling [11]. Donor bone may contain residues of functional bone growth factors. These so-called bone morphogenic proteins (BMP) are found in the organic part of the extracellular matrix and are covered by mineral, but can be exposed by bone demineralisation. This usually involves soaking bone in a hydrochloric acid solution (e.g. 0.5 or 0.6 M

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HCl) to significantly reduce the mineral content. The product of such a process is referred to as demineralised bone matrix (DBM).

Depending on other aspects of bone processing and the original BMP content, the exposition of BMP may promote 'osteoinduction'. This term is used when bone healing is initiated and maintained via stem-cell recruitment in environments lacking an adequate local population of osteoclasts and osteoblasts [12]. Due to its nature, demineralised bone matrix is often mixed with a carrier material to improve its handling properties and help retain the graft at the site of the transplantation/ grafting/ application. The safety of these carrier materials, and their effects on the essential properties of the graft, must be established.

Viable cells (e.g. chondrocytes) should be processed in a controlled and clean environment that eliminates risks of cross-contamination of tissue and culture media. This processing usually involves cell culture, and such transplants are therefore classified in the EU as advanced therapy medicinal products (ATMP) (see Chapter 30).

21.4.3 Removal of micro-organisms and virus inactivation

It is critical that the risk of transmission of microbiological agents is minimised for tissue transplants. A key factor in controlling this risk is the application of sterilisation and disinfection processes to the grafts, whether before, during or after any processing applied to the grafts. For a detailed discussion of the principles of sterilisation and disinfection, please see Chapter 8.

Sterilisation and disinfection protocols are, by their nature, aggressive processes that have the potential to damage the graft as well as to inactivate micro-organisms. It is vital therefore that, if they are to be applied, a protocol is selected that does not unacceptably impact the critical quality attributes of the graft. For example, sterilisation with gamma irradiation may damage the biomechanical properties of grafts, so should be employed with caution when applied to grafts which are expected to play a mechanical role, such as tendons or structural bone grafts. For osteochondral allografts, maintenance of donor-cell viability is crucial, so options for disinfection are limited to gentle surface decontamination.

It should also be considered that many of the routine processing techniques applied to musculoskeletal allografts, such as the use of elevated-temperature water washing, physical removal of adherent tissues and bone marrow, and the use of washes with solvents, acids or oxidising agents can also reduce microbial bioburden and so can be considered as disinfection processes.

21.5. Quality control

Quality-control tests on musculoskeletal grafts should take at least the following quality criteria into account:

a. morphology and integrity of the musculoskeletal grafts;
b. shape and size of the graft, especially for certain types of graft, for example meniscal cartilage, which require close size matching between the donor and recipient; the relevant measurements should be made using calibrated instruments when all physical processing has been completed;
c. residual moisture or available water in lyophilised or dehydrated grafts (the minimum and the maximum level to be defined according to validation studies);
d. osteo-inductive activity (*in vivo* or *in vitro*) in demineralised bone (usually demonstrated by validation rather than testing of every batch);
e. sterilisation indicators;
f. no evidence of microbiological growth;
g. number of viable cells in cell cultures (e.g. chondrocytes).

During procurement or before processing, microbiological samples should be collected to establish the initial contamination levels of tissues (bioburden) to assist in making a decision during

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10528 quarantine regarding the release of procured material for further processing. The inactivation capacity
10529 of manufacturing processes (e.g. disinfection, sterilisation) should be taken into account.
10530 Samples for microbiological testing should also be collected before or during packaging of the
10531 final product. Possible sampling techniques for microbiological testing include:

10532 • swabs;
10533 • destructive methods (e.g. biopsy or sacrificing a proportion of ground tissue);
10534 • collection of the last portion of the fluid used for washing of the tissue graft for subsequent
10535 analysis, usually following filtration.

10536 The result of the microbiological control after processing must be negative. If a positive
10537 microbiology result is obtained, the tissue should be discarded or (terminally) sterilised. Depending on
10538 the micro-organism found, a risk-assessment analysis should be done to assess the suitability of the other
10539 musculoskeletal tissues from the same donor.
10540

10541 **Table 21.2. Contaminants that should result in tissue discard if detected at any stage of processing or**
10542 **procurement**

Aspergillus spp.
Candida spp.
Clostridium spp. (notably *C. perfringens* or *C. tetani)*
Flavobacterium meningiosepticum
Klebsiella rhinoscleromatis
Listeria monocytogenes
methicillin-resistant *Staphylococcus aureus* (MRSA)
Mucor spp.
Mycobacterium spp.
Neisseria gonorrhoeae
Nocardia spp.
Pseudomonas aeruginosa or *P. pseudomallei*
Salmonella spp.
Shigella spp.
Other yeasts and fungi
Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

10543
10544 Table 21.2 lists some micro-organisms that, if detected in any culture of musculoskeletal tissues
10545 (even if detected just before processing), require the tissue to be designated as equally unsuitable for
10546 clinical use or for processing. A risk assessment including the potency of any sterilisation processes
10547 employed, and the clinical relevance of the micro-organism, should be done to analyse the suitability of
10548 the rest of the musculoskeletal tissue from same donor [13].
10549 See Chapter 10 for more detailed guidance on the principles of microbiological testing.

10550 21.6. Labelling and packaging

10551 Generic requirements are detailed in Chapter 14.
10552 Procured and processed musculoskeletal tissues are to be packaged in a way that minimises
10553 contamination risk. It is recommended that musculoskeletal tissues be at least double-packed in airtight
10554 packages or in sterile drapes as well as sterile containers. Each procured and processed tissue should be
10555 packed separately and labelled immediately.

10556 21.7. Preservation/storage

10557 After processing, grafts are stored at a tissue establishment during the quarantine period until the
10558 required test result from donor (e.g. blood cultures, serologies, autopsy and/or biopsy report) and tissues

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(e.g. microbiological test, biopsy report) are received (if required). The tissue establishment must confirm donor eligibility before releasing the graft.

Different preservation methods have been developed to maintain the biological properties of tissues for long periods of time, from processing to distribution for transplant.

21.7.1 Methods of preservation/storage

21.7.1.1 Frozen and deep-frozen

Preservation and storage of musculoskeletal tissues (including cancellous, corticocancellous and cortical bone, ligaments and tendons) by deep freezing without use of cryoprotectants is a common method. There is limited scientific evidence to justify particular temperature limits, but in general it is accepted that freezing an allograft has little impact on the mechanical properties of the tissue, and will diminish its immunogenicity. Uncontrolled freezing damages the viability of articular cartilage.

21.7.1.2 Cryopreservation

Cryopreservation is a process whereby tissues are preserved by cooling to temperatures of < -140 °C. This method is suitable for the preservation of some cell viability in cartilage. It is used for osteochondral bone grafts and for cartilage, although some centres also use it for other types of musculoskeletal tissue. Cryoprotectants – e.g. glycerol, dimethyl sulphoxide (DMSO) – are added to the medium to protect cells against freezing injury.

21.7.1.3 Freeze-drying (lyophilisation)

Lyophilisation consists in decreasing the water content of frozen tissue under vacuum through sublimation. For bone transplants, a residual moisture between 1 and 6 % is recommended. In contrast to fresh-frozen allografts, mechanical strength in freeze-dried allografts is reduced significantly, but still freeze-dried soft-tissue allograft constructs have many advantages, including limited immunogenicity, ease of graft storage, mechanical properties comparable to soft-tissue constructs, and the potential for improved biologic incorporation [14]. Freeze-drying further diminishes the immunogenicity of the graft. An alternative to freeze-drying is dehydration, where the water content should be < 15 %. Dehydration is usually performed using chemical substances.

21.7.1.4 Fresh

Storage of unprocessed tissues at hypothermic (2-8 °C) or near normothermic (~33 °C) temperatures allows maintenance of cell viability (i.e. osteochondral grafts) for a short period (1-3 months).

Different culture mediums and storage processes have been described. The main problem of fresh preservation is to have enough time to obtain test results before releasing the graft.

The tissue establishment should validate the storage method in order to guarantee a minimum rate (%) of cell viability.

21.7.2 Expiry dates

The designated shelf-life is dependent upon the packaging system (to guarantee the integrity and sterility of the graft) and the storage methods used (frozen, deep-frozen, freeze-dried, fresh, etc.).

Expiry dates should be established by the tissue establishment after a validation process. Each change in the packaging should be followed by a validation study of the packaging system and the expiry date.

21.7.3 Storage temperatures

As mentioned in section 21.7.1, different preservation methods require different storage temperatures (see Chapter 9), as shown in Table 21.3.

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Table 21.3. Storage temperatures for different preservation methods

Type of graft	*°C minimum*	*°C maximum*
Frozen	–40	–15
Deep-frozen	–80	–60
Cryopreserved	–196	–140
Freeze-dried*	+4	+30
Fresh (hypothermic)	+2	+8

*At room temperature (15-25 °C) in normal conditions of humidity

Storage time limits will be defined by expiry dates (see §21.7.2) based on the packaging and storage system and the validity of donor-selection criteria.

21.8. Distribution and transport conditions

Transportation of musculoskeletal tissues should guarantee the preservation of graft-storage conditions from tissue establishment to end user.

Transportation systems will vary, depending on the preservation method used:

a. Frozen and deep-frozen grafts can be carried using a container with dry ice or qualified cooling systems. Once the graft has been thawed, it cannot be re-frozen;
b. Cryopreserved grafts can be carried using dry-shipping containers (vapour-phase nitrogen <−140 °C). If dry ice is used for transportation of the musculoskeletal allograft, the tissue should not be returned to liquid or vapour-phase nitrogen tanks unless validated or supported by a documented scientific rationale. Once the graft has been thawed, it cannot be re-frozen;
c. Freeze-dried grafts can be carried using a container just to protect the integrity of the package system.
d. Fresh grafts can be carried using a container that ensures the defined storage temperature.

21.9. Biovigilance

The Notify Library includes many well-documented cases of adverse occurrences in the field of musculoskeletal tissue transplantation, such as:

- Bone
  - A case of human T-cell lymphotrophic virus type-1 transmission by a deep-frozen bone allograft, resulting in asymptomatic seroconversion of the recipient, is described in Record Number 587;
  - A case of human immunodeficiency virus (HIV) transmission, through frozen femoral head used in scoliosis surgery, is documented in Record Number 19. Both donor and recipient developed acquired immunodeficiency syndrome (AIDS) 40 months after transplant;
  - Several cases of serious adverse events resulting from unsuitable bone allograft release are reported in the database. Record numbers 139, 140, 141 and 142 describe bone allografts with chondrosarcoma, lymphocytic lymphoma, Paget's disease and rheumatoid arthritis respectively; all were diagnosed during histological examination of the femoral head and resulted in discarding of allografts.
- Tendon or ligament
  - In Record Number 459, a donor-transmitted invasive group-A streptococcal infection, with the diagnosis confirmed by emm gene-sequence analysis of isolates from the blood and hemi-patellar tendon tissue of the donor and recipient;
  - A case of donor-to-recipient hepatitis C virus (HCV) transmission by patellar tendon transplantation is described in Record Number 563 and confirmed by identical HCV genotype 1a and phylogenetic nucleic acid arrangement between donor and recipient;

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- An HIV type-1 transmission from a seronegative organ-and-tissue donor confirmed by the recipient's seroconversion 3 weeks post-transplantation (Record Number 581).

- Meniscus
  - Records nos. 173 and 174 describe meniscus allografts with anatomic abnormality and fracture, respectively; both were discovered in the hospital and resulted in discarding of allografts, thereby delaying treatment.

For further evaluated cases of adverse outcomes associated with musculoskeletal tissue banking, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

21.10. Developing applications

In recent years, several innovative biological products based on decellularisation of musculoskeletal tissue (especially tendons) have been developed using biotechnological sciences, based on the experience of skin-derived and cardiovascular grafts (heart valves and vessels). More information about decellularisation processes can be found in Chapter 8 and Appendix 33.

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Chapter 22. Haematopoietic progenitor cells from bone marrow and peripheral blood

22.1. Introduction

Haematopoietic progenitor cells (HPC) transplantation represents one of the most widely used forms of cell therapy, in part because haematopoiesis represents the best-known biological model of somatic stem cell and tissue differentiation. Following the first case reports more than sixty years ago, the procedure rapidly established itself as a life-saving treatment for adult and paediatric patients with a variety of malignant diseases. HPC transplantation also has a role when the haematopoietic tissue is functionally damaged by congenital or acquired disorders such as severe congenital immune deficiencies, metabolic diseases or bone marrow failure. More recently, the use of autologous HPC transplantation in combination with immuno-suppressive agents has been evaluated for patients with certain autoimmune diseases.

In its main field of application, i.e. as a component of the treatment of patients with poor-risk or advanced haematological malignancies, it is now well established that allogeneic HPC transplantation exerts its beneficial effects through the recognition of residual tumour cells in the recipient by donor-derived immune effectors (graft *versus* tumour effect, or GvT). Thus, allogeneic HPC transplantation represents a clinically useful, immune cellular therapy. Limits to the use of these therapeutic procedures are their intrinsic toxicity, dominated by (though not limited to) graft *versus* host disease (GvHD), an immune disorder in which donor-derived immune effectors recognise and harm the host's normal tissues such as skin, gut and liver, lungs and cornea.

The field has developed tremendously in the past half-century in developed countries, and now many emerging countries are establishing allogeneic and autologous HPC transplantation programmes. Work in the field has integrated medicinal and technical innovations, including the use of new immuno-suppressive agents, the use of different sources of HPC, such as bone marrow, mobilised peripheral blood and cord blood, the procurement of cells from unrelated donors and much improved supportive care for patients. During recent years, the use of unrelated, volunteer donors has dramatically increased due to the extensive improvements in HLA-typing and matching algorithms and the growth of donor registries in the majority of European and North American countries, which are united in the World Marrow Donor Association (WMDA).

Several other biotechnological advances, including stem cell selection, lymphocyte depletion, and immune effector cells activation have entered clinical practice and made haplo-identical transplantation a valid clinical choice. However, despite these advances, procurement of HPC remains relatively unchanged. Hospitals that care for recipients often obtain autologous or allogeneic HPC from hospital-based or blood establishment-based procurement and processing facilities that are located in their immediate vicinity. Each of the procurement and processing facilities works on a typically small to medium scale. In more than 50 % of allogeneic HPC transplantations, grafts from unrelated donors are used, which very often have to be imported from other countries or continents. Given the high rate of international exchange of donated HPC material, harmonisation of the practices in this field is of great benefit.

This chapter defines the additional specific requirements for procurement, processing, storage and transplantation of HPC derived from bone marrow – HPC, Marrow, known as HPC(M) – or from peripheral blood – HPC, Apheresis, known as HPC(A) – and the requirements for mononuclear cells (MNC) concentrates procured by apheresis – MNC, Apheresis, known as MNC(A) – either for

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10730 immediate use or for further development of immunocompetent cells used after or instead of HPC
10731 transplantation.
10732 The cells discussed in this chapter are regulated in the European Union (EU) under the Tissues
10733 and Cells Directive 2004/23/EC and its associated Commission directives. It should be noted, however,
10734 that if these cells are subjected to substantial manipulation (such as expansion or genetic modification),
10735 or are used in the recipient for an essential function that is different from the original function in the
10736 donor, in the EU they are then regulated as medicinal products. This means that their processing, storage,
10737 distribution and use in patients must respect the requirements of Regulation 1394/2007 on advanced
10738 therapy medicinal products ('the ATMP Regulation') as well as all other relevant provisions of the EU
10739 medicines rules.
10740 The following generic chapters (Part A) of this Guide all apply to HPC transplantation and must
10741 be read in conjunction with this chapter:
10742 a. Introduction (Chapter 1);
10743 b. Quality management, validation and risk management (Chapter 2);
10744 c. Recruitment of potential donors, identification and consent (Chapter 3);
10745 d. Donor evaluation (Chapter 4);
10746 e. Donor testing (Chapter 5);
10747 f. Procurement (Chapter 6);
10748 g. Premises (Chapter 7);
10749 h. Processing (Chapter 8);
10750 i. Storage and release (Chapter 9);
10751 j. Principles of microbiological testing (Chapter 10);
10752 k. Distribution and import/export (Chapter 11);
10753 l. Organisations responsible for human application (Chapter 12);
10754 m. Computerised systems (Chapter 13);
10755 n. Coding, labelling and packaging (Chapter 14);
10756 o. Traceability (Chapter 15);
10757 p. Biovigilance (Chapter 16).

10758 22.2. Recruitment of potential donors, identification and consent

10759 Most of the patients who could benefit from HPC transplantation do not have a genotypically identical
10760 sibling donor. The chance of having a fully HLA-matched sibling donor is 25-30 % depending on the
10761 number of siblings. In some cases, an extended family search can provide an HLA phenotypically
10762 identical donor. This can happen in cases of consanguinity due to cultural or geographical reasons and
10763 can add an extra 10 % to the likelihood of finding a matched or partially matched family donor [1]. If no
10764 HLA-matched suitable donor is found in the patient's family, an alternative HPC graft can be considered.
10765 This would include search for an unrelated donor through bone marrow or umbilical cord blood
10766 registries, and extend the search to a mismatched unrelated source or an HLA-genotypically haplo-
10767 identical relative (e.g. parents, siblings). The most common donor-selection algorithm is described in
10768 the European Bone Marrow Transplantation (EBMT) handbook (see Figure 22.1) [2]. For haplo-identical
10769 transplantation using HPC(M) or HPC(A), new promising protocols have been developed recently [3].
10770 The relevance of HLA matching is dependent on a variety of factors, including but not limited to
10771 GvHD prophylaxis, transplant conditioning, graft manipulation and clinical donor characteristics such
10772 as age, sex and *Cytomegalovirus* serostatus. Biologically, mismatched HLA can be recognised by
10773 alloreactive donor T-cells, leading both to toxic GvHD and to beneficial graft *versus* leukaemia (GvL)
10774 as counterbalancing effects [4]. Moreover, missing self-HLA on patient cells can also lead to
10775 alloreactivity by natural killer (NK) cells, a phenomenon associated with GvL but not GvHD [5].
10776 In unrelated stem-cell transplantation, it is generally accepted that the best donor is matched for
10777 8/8 HLA-A, B, C, DRB1 alleles, with every mismatch leading to an approximate 10% reduction in
10778 survival probability [6]. Mismatches at HLA-DQB1 and DPB1 are more controversial. For HLA-DPB1,

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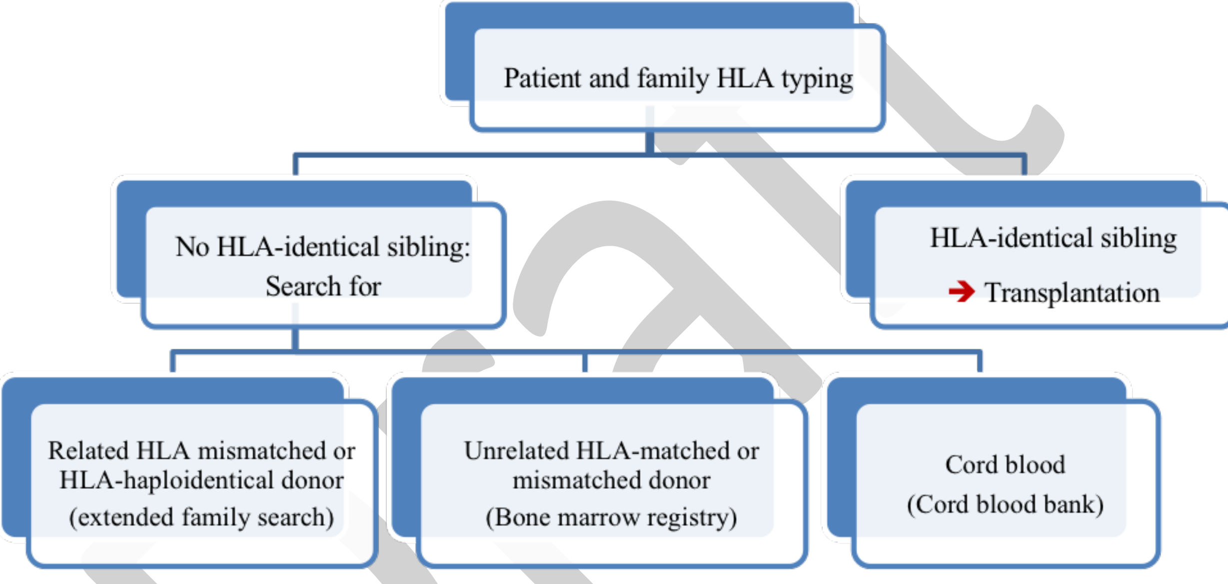
10779 the concept of permissive, clinically well-tolerated mismatches was pioneered on the basis of
10780 experimentally defined T-cell epitope groups (TCE). This led to the increasingly accepted notion that
10781 avoidance of non-permissive HLA-DPB1 TCE mismatches improves outcome and should be favoured
10782 when several 8/8 matched unrelated donors are available [7]. A mismatch at HLA-DQB1 seems to be
10783 unfavourable in the presence of other mismatches. In apparent contradiction to the dramatic effect of
10784 subtle HLA mismatches in unrelated transplantation, transplantation across several mismatches or an
10785 entire HLA haplotype are possible in cord blood and haplo-identical family donor transplantation,
10786 respectively [8, 9]. This is probably due to graft composition in the former and GvHD prophylaxis in
10787 the latter, with higher proportion of regulatory and naïve T-cells in cord blood compared to adult stem-
10788 cell sources, and an important attenuating effect of agents such as post-transplant cyclophosphamide or
10789 high-dosage anti-thymocyte globulin on GvHD in the haplo-identical setting. The important question of
10790 whether and to what extent these specific characteristics will change the landscape also of unrelated
10791 stem-cell transplantation in the future will have to be answered in prospective clinical trials under way.
10792



10793

10794 **Figure 22.1. Donor-selection algorithm**

10795 *Source:* adapted from the EBMT Handbook [2]

10796
10797 The first bone marrow donor registry was established in 1974 by the Antony Nolan Bone Marrow
10798 Trust in London. In 1988 the EBMT group with The European Donor Foundation set up the Bone
10799 Marrow Donors Worldwide Organization (BMDW) based in Leiden, Netherlands. In 2017 the WMDA
10800 took over the activities of BMDW and the NetCord Foundation and now co-ordinates the collection and
10801 listing of the HLA phenotypes and other important data of volunteer HPC donors and cord blood units.
10802 The WMDA database includes almost 33 million HPC donors and almost 750 000 cord blood units
10803 (September 2018). Since ethnic minorities are under-represented in the bone marrow registries, it is very
10804 important that donor centres work with these communities to explain the need to increase the ethnic
10805 diversity of the registry and to recruit new potential donors. Once the HLA-typing and personal data are
10806 entered onto the registry, further blood samples may be requested, leading to possible haematopoietic
10807 stem cell donation at any time in the future.
10808 The WMDA, an organisation of representatives of HPC donor registries, cord blood banks, other
10809 organisations and individuals with an interest in HPC transplantation (www.wmda.info) plays an
10810 important role in the field of HPC transplantation from unrelated donors, providing a forum for
10811 discussion and guidelines on the more critical aspects of the procurement and clinical use of HPC.

10812 Moreover, the WMDA offers to the donor registries an accreditation programme according to
10813 internationally accepted standards.
10814 The main role of the registries is to facilitate interactions between HPC transplant centres and
10815 donor centres. In most of the countries involved, the search processes – including HLA confirmatory
10816 typing requests and donor selection – are operated using EMDIS (the European Marrow Donor
10817 Information System), an international computer network which allows fast and direct communication
10818 between registries (see Appendix 28).
10819 Emergency rescue procedures should exist to limit consequences related to unforeseen
10820 unavailability of a donor (e.g. acute illness, accident, failed mobilisation). This could be: search for a
10821 cord blood unit, a haplo-identical donor or an autologous transplantation with previously cryopreserved
10822 autologous HPC.

10823 **22.2.1. Donor evaluation**

10824 *22.2.1.1. Allogeneic donor*
10825 One of the fundamental principles of volunteer stem cell donation is the right of the potential
10826 donor to proceed to donation with a minimum of extraneous influences and pressures. Protection of the
10827 donor's identity has to be guaranteed. Therefore, all the activities related to the donor's physical
10828 examination and collection of personal data must be performed in a dedicated and restricted area, as the
10829 access to all the donor information (medical and personal) must be protected and limited to authorised
10830 individuals [10]. Recommendations on assessing the medical suitability of adult stem cell donors are
10831 shown in Table 22.1.
10832 For the evaluation of allogeneic donors, written criteria – in the form of standard operating
10833 procedures (SOPs) – should exist. Criteria must take into consideration not only the recipient's safety
10834 but also the donor's safety. Specific recommendations should be in place, especially for related donors
10835 who would not be eligible as unrelated donors due to age (e.g. young donors < 18 years, elderly donors
10836 > 60 years) or specific health issues [11-14]. The risk of donation should be evaluated and documented.
10837 To avoid a conflict of interest, the physician who evaluates the donor should be independent of the
10838 transplantation team. In any case, donor evaluation must be completed before the patient starts with the
10839 preparative regimen and, in cases involving HPC from the peripheral blood, before the donor receives
10840 the first dose of the mobilisation agent. To ensure the security of donor and recipient, a physician
10841 involved in the HPC(A) procurement procedure must be available during the procurement period, from
10842 the beginning of G-CSF (granulocyte-colony stimulating factor) injections to the post-procurement
10843 period.
10844

10845 **Table 22.1. Recommendations on assessing the medical suitability of adult stem cell donors [15]**

Assessment stage	*Method*	*Topics to consider*	*Specific for this stage*
Recruitment/ registration (unrelated)	History/questionnaire	Malignancy Autoimmune disease Cardiovascular disease (or a combination of risk factors therefor) Chronic disease (pulmonary/neurologic/hematologic/ serious allergies) Relevant medical history (malignancy, thrombo-embolic disease etc.) Risks of infectious diseases (behaviour) Inherited/genetic disease	Look for permanent diseases or behaviour that have a clear donor risk or unacceptable recipient risk and that are relatively easy to assess
During selection stage (unrelated)	History/questionnaire Blood tests for infectious disease markers (HIV, hepatitis B, hepatitis C, HTLV, syphilis, *Cytomegalovirus*)	Update history of topics above, and also: Risks of infectious diseases – behaviour, (medical) invasive procedures, (planned) travel	Identify contraindications for one of the two collection methods Provide information about (possible)

		(Planned) medical procedures (including blood transfusion, dentist, vaccination, tattoo etc.) Serious psychosocial or psychiatric disease with impact on availability/ capacity to go through donation procedure Medication Non-prescription drug use Height and weight Blood pressure Pregnancy, pregnancy planning, breastfeeding Back problems, chronic pain	transmittable disease to the transplant centre Provide information to the transplant centre about any availability issues
Prior to HLA-typing (related)	History/questionnaire	As above	Identify contraindications before concluding that the relative donor is the best match; may save time and disappointment
During work-up/ PE (related and unrelated)	History including full tract history; Complete physical examination; Laboratory tests: ● Infectious disease markers: HIV-1,2 antibody, p24 antigen, HIV NAT, hepatitis B surface antigen and core antibody, hepatitis B NAT, hepatitis C antibody, hepatitis C NAT, validated serological testing algorithm for syphilis, on indication/per request transplant centre: HTLV I+II antibody, Chagas, Zika, Malaria, West Nile Virus, etc.; ● Full blood count; ESR; if indicated: coagulation screen; blood film; hemoglobin electrophoresis; ● ABO and Rh typing, screening for red blood cell and HLA-antibodies; ● Biochemistry: Urea, creatinine, electrolytes, liver enzymes, LDH, ferritin; random glucose; β-HCG (for females of child-bearing age), protein electrophoresis; Chest X-ray; Electrocardiogram	As during selection stage, in addition any signs of undiagnosed disease	Emerging infectious disease: check latest infectious disease epidemiology maps (CDC, ECDC)

10846

10847 Criteria for donors should include, in addition to general requirements, at least:

10848 a. suitability for anaesthesia (for procurement of bone marrow);

10849 b. assurance of adequate venous access;

10850 c. additional test if appropriate (e.g. in cases of family history or elderly donor);

10851 d. exclusion criteria (see Chapter 4);

10852 e. policy for making decisions in cases of ‘only one’ donor but who does not meet eligibility criteria

10853 (e.g. only one suitable donor but with risky behaviour);

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f. a donor advocate should be available to represent allogeneic related donors who are mentally incapacitated or not capable of full consent.

Donors with history of malignant disease (excluding haematological malignancies) after a minimum disease-free period of 5 years can be considered as suitable for sibling donation after careful assessment of the risk for malignant disease transmission [11].

It is up to the discretion of the transplant physician whether to accept donors with previous or existing infectious diseases (e.g. hepatitis B or others) if no other donor is available, based on careful risk evaluation. The specificity of allogeneic HPC transplantation lies in the fact that, for the vast majority of patients, the HPC graft is infused immediately after procurement. Thus, safety is reliant mostly on stringent evaluation of donors, which can be performed appropriately only if all needs are fully anticipated. In case of a risk with regard to patient or product safety, a formal acceptance of that risk should be signed by the transplant physician and the patient or their legal representative (urgent medical need).

The increasing age of recipients of allogeneic HPC transplants is related not only to the increasing age of the population but also to the introduction of less toxic conditioning regimens, allowing elderly patients and patients with comorbidities to undergo HPC transplantation. Even if the age limit to donate is well-defined for unrelated donors, and is over 18 and under 55-60 years for most international registries, these limits do not strictly apply for related donors. The decision process to collect from young (< 18 years) and elderly donors (> 60 years) must include an accurate risk assessment and appropriate informed consent of the donor stating that risk [11-13].

Decisions with regard to donor safety should be the responsibility of the independent donor physician. Some donors will present with comorbidities (discovered or not) during evaluation. If these comorbidities result in contraindication of the person for HPC donation, the physician who decided to contraindicate this person must ensure that a correct medical follow-up will be initiated in accordance with the medical condition of the rejected donor.

If the comorbidities found during evaluation allow HPC donation, the physician in charge must manage these comorbidities during the entire donation process, including specialist consulting as needed.

There should be a written plan to care for paediatric donors, donors with comorbidities and elderly donors during selection [11-14].

22.2.1.2. Autologous donors

For autologous donors, suitability criteria for HPC donation are less strict than for allogeneic donors. For evaluation, written criteria – i.e. SOPs – should exist. Criteria must take into consideration the patient's safety. Criteria should include threshold values of the complete blood count before starting bone marrow procurement or leukapheresis. The risk of donation should be evaluated and documented. A donor advocate should be available to represent autologous donors who are mentally incapacitated or not capable of full consent at the time of HPC mobilisation and donation (e.g. for patients with primary CNS lymphoma).

Infectious disease markers in autologous donors should be tested as required by applicable laws and regulations (see Chapter 5). Autologous donors can donate even if results of the required tests are reactive or positive or other risk factors have been identified in patient's medical history as long as potential cross-contamination during HPC procurement, processing and storage can be prevented.

22.2.1.3. Specific considerations for paediatric donors

If minors are being considered as allogeneic HPC donors, in addition to the criteria shown in Chapter 3, national regulations should be followed. The use of haematopoietic growth factors and insertion of a central venous line are not recommended. Procurement methods for paediatric donors should employ appropriate adjustments for age and size to the procedure.

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Children should become donors only in very specific circumstances, as family donors only and never through public registries (see Chapter 3).

22.2.2. Informed consent

Informed consent is required for an allogeneic and also for an autologous donation. In cases of autologous donation, the informed consent should include terms and conditions for the HPC storage and disposal. Volunteer HPC donors joining a bone marrow registry express their commitment to donate, but they must nevertheless sign a formal consent before the HPC procurement. General considerations are shown in Chapter 3. Discard of allogeneic grafts from unrelated donors should comply with written agreements with donor registries.

22.3. Procurement

22.3.1. Haematopoietic progenitor cell graft sources

HPC(M) and HPC(A) are obtained from living donors only, either from the recipient patient (in the case of autologous transplantation) or from a fully or partly HLA-matched allogeneic related or unrelated donor. The choice of the donor is based on the best HLA matching, and other factors like age, gender, *Cytomegalovirus* status, ABO compatibility and NK alloreactivity.

For autologous purposes, nowadays HPC are obtained almost exclusively from peripheral blood stem cells HPC(A) and used to accelerate haematopoietic recovery after high doses of chemotherapy. In the allogeneic setting, the HPC graft source depends on the age and size of the donor and recipient – i.e. paediatric or adult donor, since some countries do not support G-CSF administration and apheresis in paediatric sibling donors – and the kind of disease (malignant or non-malignant), as well as the transplant protocol (myeloablative, reduced intensity, T-cell replete or deplete haplo-identical transplantation). HPC(M) are still the preferred source in allogeneic paediatric transplantation from compatible related or unrelated donors.

Indications for HLA-mismatch transplantation and selection of 'alternative' donors – in the sense of donors without a 10/10 HLA match with the recipient (considered as 'standard' donors) – are constantly implemented as they are evaluated in the context of biomedical research or registry studies. To date, prospective clinical studies comparing all sources of alternative HPC donors in different clinical settings are still ongoing. Hence, transplantation programmes should carefully follow their own local algorithms defining the 'best donor' for each patient according to the different situations, and use these algorithms to guide the donor choice.

Particular attention should be paid in HLA-mismatched donor selection (parents, brothers/sisters, adult children, or other family members). The criteria of choice should be explained in advance to the patient and potential family donors.

22.3.2. Procurement procedures

The majority of HPC are provided using two technologies: procurement of bone marrow and apheresis. The advantages and disadvantages of these technologies are shown in Table 22.2.

A risk tool (see also Chapter 6) should be used to evaluate the contamination risk factors during procurement, as shown in Table 22.3.

For HPC procurement and associated procedures – e.g. central venous (CV) line placement – written procedures must be established and reviewed regularly, with evidence of continued training of the staff. The International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration were developed by two organisations, JACIE (the Joint Accreditation Committee–ISCT & EBMT) and FACT (Foundation for the Accreditation of Cellular Therapy), and provide minimum guidelines for transplant, procurement and processing facilities. These standards can serve as guidance [16].

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10946 Severe adverse reactions can occur in donors with the administration of the mobilisation agents,
10947 and during and after allogeneic HPC donations. Hence, careful and documented training of clinicians
10948 and involved healthcare professionals caring for donors is needed, as is appropriate follow-up of donors.
10949 In all cases, safety of the donor is the major concern, meaning that the pre-donation work-up should be
10950 particularly meticulous [10, 11, 17-20].

10951 **Table 22.2. Advantages and disadvantages of methods of haematopoietic progenitor cell procurement**

Procurement method	*Advantages*	*Disadvantages*
Bone marrow procurement	Donor: • single procurement; • use of cytokines (mobilisation agents) not necessary Recipient: • less chronic GvHD	Donor: • general or epidural anaesthesia • invasive procedure • considerable risk of morbidity (associated with anaesthesia, procurement method, mobilisation agents if used) • potential tissue damage/infection at procurement site • possible need for blood transfusion Recipient: • slower engraftment of neutrophils and platelets • potential graft contamination with skin contaminants • possible contamination with tumour cells in autologous HPC procurements
Peripheral blood apheresis	Donor: • no anaesthesia Recipient: • faster engraftment of neutrophils and platelets • potentially less contamination of autologous product by tumour cells	Donor: • procurement may take more than one day (i.e. several procedures may be needed) • may require placement of a central venous catheter for procurement (risk of haemorrhage, embolism, pneumothorax/haematothorax and infection) • potential loss of platelets • considerable risk of morbidity (associated with mobilisation agents and apheresis technique including anticoagulation) Recipient: • increased risk of chronic GvHD

Source: EBMT [14].

10952

10953 **Table 22.3. Risk of contamination in HPC and MNC procurement**

HPC Sources	*Air quality of procurement area*	*Procurement system*	*Microbial spectrum*	*Risk of microbiological contamination*
HPC, Marrow	classified	open[1]	skin contaminants	low
HPC, Apheresis	non-classified	closed[2]	skin contaminants (secondary contamination) venous catheter contaminants (patient)	very low
MNC, Apheresis	non-classified	closed[2]	skin contaminants (secondary contamination) venous catheter contaminants (patient)	very low

[1]Open system: A procurement system that exposes the cells to the environment. The environment has to be controlled to minimise the risk of graft contamination (e.g. operation theatre).

[2]Closed system: A procurement system with equipment designed and operated such that the cells are not exposed to the environment. If closed systems are not operated accordingly (e.g. second puncture of a peripheral vein without appropriate disinfection or without clamping) the product may be exposed to the environment.

10954

10955 *22.3.2.1. HPC from bone marrow*

10956 Procurement of bone marrow is an aseptic process that should be undertaken in an operating
10957 theatre by appropriately trained personnel. Special attention should be paid to the training of clinicians,
10958 to written criteria for required competence and experience in bone marrow procurement and aftercare

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of the donor, and to vigilance and surveillance of donors as well as of recipients. There must be provision for counselling of donors and for their routine post-donation follow-up.

Bone marrow for therapeutic use is obtained through multiple punctures, usually from the posterior iliac crests. When absolutely necessary, the anterior iliac crests can also be used. The sternum is not considered an appropriate method of bone marrow procurement. Punctures are usually undertaken under general anaesthesia. Epidural anaesthesia may be considered. A pre-anaesthesia visit of the donor is mandatory before procurement of bone marrow.

For procurement of bone marrow, written procedure(s) should be established, including at least:

a. disinfection technique;
b. preparation of media and materials used to obtain bone marrow;
c. puncture technique;
d. provision of containers for procured bone marrow;
e. monitoring of the volume of the procured bone marrow;
f. irrigation of procurement syringes;
g. bone marrow filtration~~;~~
h. quality controls (e.g. TNC counting, sterility, etc.);
i. labelling.

Bone marrow grafts contain bony spicules, fat and clots that should be filtered during procurement or processing. The bone marrow total nucleated cell number (TNC) is used to determine the adequacy of the procurement. The recipient's body weight and type of post-procurement manipulation determine the target TNC and volume of bone marrow to be procured. The procurement and anaesthesia times should not exceed 120 and 150 minutes, respectively. A maximum volume of 20 mL of bone marrow/kg donor weight should be procured. Procurement teams should not aspirate a volume more than 5 mL at each aspiration to avoid dilution of the bone marrow with blood [2]. The minimum target for autologous transplantation without graft manipulation is 2×10^8 TNC/kg recipient body weight. The target dose for most allogeneic transplantations is 2-3.5 $\times 10^8$ TNC/kg recipient body weight. To increase TNC counts, mobilisation agents (e.g. rhG-CSF) can be used in autologous and allogeneic donors.

Adverse reactions related to HPC Marrow procurement are associated with anaesthesia, pain at aspiration sites, bruising and, rarely, local infection.

Blood cultures should be taken from bone marrow donors in presence of fever to investigate a possible microbial contamination of the procured HPC graft.

A 24-hour blood component donor-support protocol, including the provision of *Cytomegalovirus* antibody-negative (or equivalent), irradiated and leukocyte-depleted blood components, should be available. However, all efforts should be made to manage allogeneic donors in such a way as to minimise the need for blood transfusions. Autologous red blood cell (RBC) donation before bone marrow procurement can be considered, but potentially induces iron deficiency, because the time from final selection of donor to procurement can be short. Donation of autologous blood should be over a reasonably long period before procurement but ≥ 1-2 weeks [21]. Autologous blood must be taken in a blood-collection facility that meets applicable national/international requirements. When autologous RBC donation is considered, a protocol describing the transfusion trigger should be in place. Iron supplementation before and after bone marrow donation is recommended if possible, reducing the need for RBC transfusion.

Procurement procedures in paediatric donors should be adjusted according to donor age and size [16].

22.3.2.2. HPC from peripheral blood

In both autologous and allogeneic settings, HPC from peripheral blood should be procured in an apheresis facility by health professionals who have appropriate experience in care for patients with haematological or oncological diseases, HPC mobilisation and therapeutic apheresis. Special attention should be paid to paediatric patients and the specific circumstances pertaining to apheresis in young

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patients, whose weight (often < 20 kg) places them at risk of haemodynamic changes, both on commencement and during the procedure. Expertise to carry out apheresis is of particular importance for small children (< 20 kg) for autologous procurement (which is usually indicated in solid tumours or haematologic malignancies); the transplantation programme must maintain trained and experienced personnel for apheresis in paediatric units.

Before each apheresis procedure, the donor (autologous and allogeneic) should be evaluated. At least the following studies should be carried out:

a. complete blood count (including platelet count) ≤ 24 h before procurement;
b. vital signs and temperature;
c. blood cultures in presence of fever to investigate a possible microbial contamination of the procured product;
d. update of medical history.

22.3.2.2.1. Allogeneic donors

Mobilisation of HPC to peripheral blood before allogeneic procurement is ensured by recombinant human granulocyte-colony stimulating factor (rhG-CSF), which is administered to healthy adult donors in order to mobilise HPC from bone marrow into peripheral blood. The side-effects and risks associated with the procedures must be discussed with the donors. In particular, the donors must be informed about all aspects of rhG-CSF administration, including known short- and long-term effects, and given the opportunity to ask questions. Immediate and severe side-effects associated with rhG-CSF administration are rare (see Table 22.4), and raise the issue of inpatient *versus* outpatient administration. Donors who live far away from the transplant centre will require administration at home. However, because of a potential risk of allergic reaction, the first dose of rhG-CSF should be given under the supervision of trained healthcare professionals, and the donor should be followed up for at least 30-60 minutes. The transplantation programme, or the physicians in charge of mobilisation and procurement of HPC from the donor, should be informed in detail of the necessary measures to be taken in case severe adverse reactions (SARs) occur, especially for anaphylactic shock, spleen rupture, capillary leakage and acute hepatitis.

Routinely, HPC(A) procurement takes place on day 5 after 4 days of rhG-CSF administration. In cases of very low $CD34^+$ cell numbers, rescue strategies should be established (e.g. 'immediate' bone marrow procurement, administration of additional agents like HPC binding inhibitors).

Approximately 5-10% of the donors may be asked to provide a subsequent donation of HPC or MNC concentrates to the same patient. The frequency of second donations seems to be higher for HPC(M) donors, and it may increase for the application of new therapeutic strategies. The interval between donations, for the same or for a different recipient, should be established by individual registries or transplant centres on the basis of the risks to the donor and the patient. However, these limits do not strictly apply to related donors.

22.3.2.2.2. Autologous donors

Cell mobilisation before autologous procurement is ensured by administration of various types of mobilisation regimens. RhG-CSF is the usual haematopoietic growth factor used to mobilise progenitors and can be administered alone or in combination with chemotherapy or other agents (e.g. HPC binding inhibitors such as Plerixafor, immunostimulants).

Circulating levels of $CD34^+$ cells guide commencement of apheresis. The number of cells required varies with the size of the patient and number of transplantations indicated (double grafting is indicated for some diagnoses). Procurement centres should have protocols that can determine the optimal number of cells to be procured, taking into account the patients' well-being during and after procurement, as well as their needs as future recipients.

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Table 22.4. Very common adverse reactions associated with HPC mobilisation (> 10 %)

Agent	*Adverse reaction*
Rh-Granulocyte-colony stimulating factor (rhG-CSF)	bone pain musculoskeletal pain thrombocytopaenia hyperleukocytosis transitory elevation of levels of liver enzymes elevation of levels of lactate dehydrogenase headache asthenia
Haematopoietic progenitor cell binding inhibitors (Plerixafor)	diarrhoea nausea reaction at injection site

Source: EBMT [14].

In the selection process of the mobilisation agent, the World Marrow Donor Association document *Recommendation for recombinant human G-CSF (G-CSF) that stem cell donor registries can use – The use of biosimilar G-CSF* [22] should be consulted. The relevant mobilisation agent should be used in accordance with the latest approved Summary of Product Characteristics.

22.3.2.2.3. Apheresis procurement yield

Processed blood volumes vary, depending on the procurement protocol and cell separator. The target number of $CD34^+$ cells should be set before starting apheresis according to institutional protocols. The target will vary for autologous and allogeneic donations, and will depend on clinical need and regulations (as well as best available professional practices). Target dose of $CD34^+$ cells for a single autologous transplantation ranges from a minimum of 2×10^6 $CD34^+$ cells/kg recipient weight to a more preferable 5×10^6 $CD34^+$ cells/kg recipient weight. The ability to achieve this goal is dependent on the underlying disease of the patient, therapy and mobilisation protocol.

The target for allogeneic donations is higher, because of the longer time to engraftment of neutrophils and platelets associated with allogeneic transplantation; HPC(A) doses above 4×10^6 $CD34^+$ cells/kg might be needed, especially when CD34 enrichment (a loss of $CD34^+$ cells is expected) or T-cell depletion methods are used. In addition to optimising HPC procurement, apheresis should ensure that procured cells have minimal contamination with neutrophils that could compromise subsequent processing steps or contribute to side-effects in recipients. HPC from apheresis contain small volumes of RBC (haematocrit < 5-10 %) so the risk of donation-related anaemia is very low [13]. Depending on the device used for procurement of HPC(A), the donor may experience a marked loss of platelets, in some cases resulting in post-donation values < 100 G/l. Under such circumstances a consecutive donation has to be carefully considered. Other risks related to the procurement method are given in Table 22.3.

The targeted cell dose could be reached in one or more apheresis procedures.

Some Health Authorities do not permit the use of G-CSF in paediatric donors and so bone marrow procurement might be employed.

22.3.2.3. MNC from peripheral blood

To enhance immune responsiveness after HPC transplantation specific mononuclear cells are being used and/or evaluated in clinical trials. However, these immunocompetent cells are also used in patients who had not undergone an allogeneic or autologous HPC transplantation previously. As starting material, MNC concentrates are procured from an unstimulated donor. This donor can be a former HPC donor (i.e. donor lymphocytes infusion), a third party donor or the patient (autologous donor).

Donor lymphocytes infusions (DLI) can be administered to the selected patient after allogeneic HPC transplantation, either prophylactically to augment the anti-tumour immune response (following reduced-intensity conditioning protocols), in cases of mixed chimerism or of relapse of an underlying

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disease (mostly myeloid malignancies). The goal of this therapy is either to induce complete donor chimerism or a remission of the patient's malignancy by a process called graft *versus* tumour (GvT) effect.

The following cells are being used and/or evaluated in clinical trials (see also Chapter 32):

a. DLI to enhance immune surveillance against infections in patients with poor immune recovery experiencing relapsing/resistant viral infections;
b. T regulatory cells (Treg) for the prevention and control of GvHD;
c. natural killer (NK) cells as GvT effectors by alloreactivity of killer Ig-like receptors (KIRs) in donor–recipient direction;
d. viral- and fungal-specific T-cells for the treatment of several infections (e.g. *Cytomegalovirus*, Epstein–Barr virus, *Adenovirus*, *Aspergillus*);
e. vaccination with peptide-loaded dendritic cells (DC) for induction of tumour-specific T-cell responses for treatment of metastatic disease transplantations, or for treating GvHD;
f. mesenchymal stem cells to enhance engraftment in allogeneic and autologous HPC transplantations, or in treatment of GvHD;
g. autologous or allogeneic chimeric antigen-receptor (CAR) T-cells.

22.3.3. Temporary storage and transportation to the tissue establishment

Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, HPC(A) and HPC(M) should be stored in non-frozen conditions before processing and infusion or during transportation. Survival of HPC stored in a non-frozen state is dependent on the concentration of leukocytes (TNC), buffering capacity of the HPCs and anti-coagulant in the graft, product volume and storage temperature. Cell viability decreases and the risk of bacterial growth increases during storage at room temperature as well as in refrigerators (see Chapter 9, also **Table 7.2**). Therefore, maximum storage in the non-frozen state should be ≤ 72 h. In cases where HPCs have to be cryopreserved, this should be done as early as possible (i.e. within 48 h after procurement) to avoid cell loss and reduced viability during processing. The facility should undertake a validation study of the storage and transport conditions.

The same applies to MNC(A) concentrates procured for DLI.

22.4. Processing of HPC

Processing of minimally manipulated HPC is intended to provide appropriate conditions for preservation and storage or to improve the risk–benefit ratio of autologous or allogeneic HPC transplantation [2,16]. It does not affect the main biological property of the procured cells, which is to support the marrow re-populating ability (MRA) and the establishment of haematopoietic chimerism in a myelo-ablated or immuno-suppressed recipient in allogeneic transplant.

Generic requirements for processing facilities, together with standards, are described in Chapters 7 and 8.

The specificities of processing HPC are shown in Table 22.5 (see also Chapter 8, **Table 8.2** on the risks of airborne contamination). While HPCs are exposed to the environment, processing should be performed in a laminar-flow cabinet of GMP Grade A with background environment to at least equivalent to GMP Grade D as required by the Directive 2006/86/EC. Considering the factors detailed in Table 22.5, it is appropriate that HPC processing takes place in a microbiologically and climate-controlled environment (control of temperature, ventilation, air filtration) with validated cleaning and disinfection. The same requirements apply for autologous or allogeneic donations.

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11138 **Table 22.5. Factors influencing the air-quality specification for HPC processing**

Criterion	*Haematopoietic progenitor cell-specific*
Risk of contamination of tissues or cells during processing	Cryopreservation or selection of certain subpopulation of HPC is mostly done in closed systems. Processes that are closed need a less stringent specification than those that involve hours of open processing. The sterile barrier can be compromised in a moment after adding cryoprotectant, monoclonal antibodies or other solutions by sterile spikes.
Use of antimicrobials during processing	Use of antimicrobial agents during HPC processing is not applicable. Nevertheless, in some cases, even HPC contaminated by bacteria can be used (unique matching, life-saving treatment). In such cases, the recipient is protected with antimicrobial agents.
Risk that contaminants will not be detected in the final tissues or cells due to limitations of the sampling method	Obtaining adequate volumes of the sample is the main obstacle in final microbiological control, especially in CD34 selected grafts. Samples can be taken from the cells or residual components after processing, depending on the product volume. Procedures for microbiological detection should be validated for inadequate quantities of sample.
Risk of transfer of contaminants at transplantation	Nature of transplant (blood cells), minimal processing and the fact that there are no applicable decontamination procedures make these cells high-risk for transfer of contaminants at transplantation. However, application of the transplant is by infusion, during which the transplant is not exposed to the environment. Hence, the risk of contamination during transplantation is minimised. A possible risk is the thawing procedure. Thawing in a water-bath bears a risk of contamination.

11139
11140 For all processing steps, written procedures must be established and reviewed on a regular basis
11141 with evidence of continued training of the staff. The current FACT–JACIE International Standards for
11142 Hematopoietic Cellular Therapy Product Collection, Processing, and Administration also apply to
11143 processing facilities.

11144 **22.4.1. Volume reduction**
11145 Volume reduction is either a preparatory step to further processing (including cryopreservation
11146 and storage) or a means to reduce the volume of the infused cells and, thus, prevent recipient side-effects
11147 relating to volume overload in the transplanted patient. Various centrifugation-based techniques can be
11148 used that are validated at the site. Cell loss associated with volume reduction must be evaluated and
11149 expected recoveries defined.

11150 **22.4.2. Red blood cell depletion**
11151 Red blood cell depletion is a critical step in cases where there is major ABO incompatibility
11152 between a donor and a recipient in the allogeneic setting (HLA identity does not preclude the existence
11153 of major or minor ABO incompatibility). Red blood cell depletion is almost exclusively performed if
11154 bone marrow is used as the HPC graft source, whereas HPC(A) are usually not red blood cell depleted.
11155 Various techniques for blood cell depletion are available, including buffy-coat centrifugation or
11156 apheresis cell separation. The efficiency of the technique must be monitored by measuring the residual
11157 content of red cells, which should be as low as possible. Similarly, the cell loss associated with such
11158 procedures must be evaluated and the expected recoveries and amount of acceptable residual red blood
11159 cells must be defined.

11160 **22.4.3. Plasma removal**
11161 Plasma removal represents a critical step in cases with minor ABO incompatibility between a
11162 donor and a recipient in the allogeneic setting (HLA identity does not preclude the existence of major
11163 or minor ABO incompatibility). The necessity of plasma removal in case of minor ABO incompatibility
11164 can be judged using a titration of anti-A and anti-B antibodies in the donor blood during the period that
11165 precedes the donation. In minor ABO incompatibility, where anti-recipient-RBC antibodies are 1:256 or

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11166 greater, plasma should be removed, especially from bone marrow grafts [2] Plasma removal is usually
11167 done by centrifugation of the procured cells. The cell loss associated with such procedures must be
11168 evaluated and expected recoveries must be defined.

11169 **22.4.4. Cryopreservation, thawing and infusion**

11170 *22.4.4.1. Cryopreservation of HPC*

11171 The purpose of cryopreservation is to preserve HPC in such a way as to ensure their viability and
11172 potency. To minimise the volume infused, peripheral blood and bone marrow may be concentrated
11173 before cryopreservation. The volume of cell suspension per bag should be adjusted by the body weight
11174 of the patient. A maximal daily dose of dimethyl sulphoxide (DMSO) of 1 g/kg body weight, which is
11175 equivalent to an infusion of 10 mL/kg of cells in 10 % DMSO solution, should not be exceeded. Special
11176 attention should be paid if the recipient is a small child and if a patient has impaired renal or liver
11177 function.

11178 Cryopreservation is used systematically in the autologous setting. In the allogeneic setting, cell
11179 procurement from the donor is usually synchronised with administration of a conditioning regimen to
11180 the recipient and direct infusion of the HPC product (within ≤ 48-72 h after procurement) without
11181 freezing. However, sometimes allogeneic HPC are being cryopreserved for logistical reasons, such as
11182 unavailability of the donor at the scheduled date of transplantation (procurement in advance),
11183 professional constraints, unforeseen changes in transplantation schedules or over-collection of stem
11184 cells. HPC should be cryopreserved as soon as possible. Shelf life of HPC without cryopreservation is
11185 acceptable up to 72 h. However, cell viability decreases if cells are frozen at the end of their shelf life.

11186 The cryopreservation method for HPC(A) or HPC(M), once RBC and plasma is depleted from
11187 the latter, is the same. The method involves addition of 5-10 % DMSO to a suspension of HPC and
11188 protein-rich medium, with or without dextran or hydroxyethyl starch (HES). Immediately after DMSO
11189 addition, HPC are cooled at −1 °C to −2 °C per minute. For most therapeutic cells, the cooling rate is
11190 controlled by a controlled-rate freezer in which vapour-phase liquid nitrogen is pumped into the freezing
11191 chamber facilitating a sudden temporary drop of the temperature in order to compensate for the thermal
11192 release caused by the solidification of the suspension. Although this is the recommended method for
11193 cryopreservation of therapeutic cells, other methods may be used (e.g. freezing in a mechanical freezer)
11194 as long as they result in acceptable post-thaw viability and potency. The final phase of cooling in a
11195 controlled-rate freezer is usually quicker, with the temperature drop adjusted to 5 °C/min. When the
11196 mixture has reached approximately −100 °C to −120 °C, it is transferred to a storage container. Methods
11197 to minimise the risk of contamination or cross-contamination must be in place (e.g. secondary bag, liquid
11198 nitrogen vapour phase). Temperature fluctuations may result in a loss of viability. The validation of
11199 cryopreservation procedure must include evidence that the storage temperature is adequate to preserve
11200 the grafts.

11201 Once frozen, HPC should be stored in vapour-phase liquid nitrogen or in liquid nitrogen at
11202 < −140 °C. Variations in cryopreservation methods include the concentration of frozen cells, the amount
11203 and source of plasma protein and the cooling rate. The method chosen must be validated.

11204 Maximal shelf life has not been defined for cryopreserved HPC. HPC(M) or HPC(A) have been
11205 transplanted successfully even 11 years after cryopreservation. A stability programme for cryopreserved
11206 grafts should be implemented in order to evaluate viability and potency at different storage durations.

11207 *22.4.4.2. Cryopreservation of MNC*

11208 Processing of MNC(A) mainly involves adjustment of volume and cell number according to the
11209 clinical protocol used. The number of MNC and specifically the number of $CD3^+$ T-cells is determined
11210 by flow cytometry analysis, and further characterisation of T-cell subpopulations may be undertaken
11211 according to special requirements and needs.

11212 For DLI, frequently a T-cell dose of 1×10^6 $CD3^+$/kg body weight of the recipient is the starting
11213 dose, and then further treatments with escalating doses may be used. The tissue establishment should

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validate the freezing technique in order to establish the expected level of viable T-cells after thawing. Some protocols also include cell-selected preparation.

22.4.4.3. Thawing and infusion

HPC(M) and HPC(A) can be thawed at the bedside or in a processing facility. Several studies have demonstrated that the occurrence of adverse reactions during HPC infusion is related to the amount of DMSO and/or cell debris in the product, and some centres remove DMSO after thawing prior to infusion. This procedure is performed in the processing facility by manual centrifugation or by automated washing in closed systems using specific equipment. Despite the progress that has been achieved in the development of new washing methods, such as membrane filtration and new devices, automated washing is still performed only in few transplant centres because of the associated risks: cell clumping, cell loss, osmotic injury, contamination and the high cost.

Hence, washing of HPC(A) and HPC(M) must be reserved only for patients at a high risk of adverse reactions. Good practice recommends (if possible) not exposing all cells to the risk of washing procedures at once unless there is a validation that demonstrates the maintenance of morphological and functional characteristics of the cells.

HPC should be infused immediately after thawing and as fast as possible, at approximately 5-20 mL/min using standard transfusion sets, although this interval may be longer if the HPC are washed. Leukoreduction filters must not be used.

22.4.5. Cell-selected preparations

Specific Conformité Européenne (CE)-marked devices are available to select $CD34^+$ cells from bone marrow or peripheral blood on the large scales needed for clinical transplantation. The use of such medical devices requires adequate training for personnel involved in these procedures. There must be written criteria – i.e. SOPs – for cell-selection preparations and the criteria must be reviewed on a regular basis with evidence of continued training of the staff. The method chosen must be validated and ensure a sufficient purity and viability for a safe transplant and sustained engraftment.

22.4.5.1. T-cell depletion and depletion of alloreactive immune effectors

T-cell depletion is associated with positive (i.e. GvHD prevention) and negative (i.e. prolonged immuno-suppression) consequences that prevents its adoption in routine clinical practice, and it is rarely used in HLA-identical or HLA-matched transplantation. This is because the advantages of reducing GvHD are offset by associated increases in relapse rates and graft failures. Indications for T-cell depletion depend on the clinical protocol, such as use of haplotype-mismatch donors and transplantation for non-malignant diseases. In these situations, it is important that T-cell depletion is as extensive as possible.

Accurate determination of the residual T- and B-cell content is mandatory. The highest acceptable dose of residual T- and B-cells must be defined in advance by the medical team in charge of the recipient. CD34-positive immunoselection can also be considered a T-cell depletion method because, as in standard CD3 depletion, almost all T-cells are eliminated, including the T-cell receptor (TCR) gamma/delta-positive T-cells not involved in GvHD and exerting anti-leukaemic activity, as demonstrated by several authors. A specific depletion of TCR alpha/beta-positive cells spares the gamma/delta T-cells and is more beneficial over the depletion of all T-cell subsets. Combining this with a $CD19^+$ B-cell depletion for preventing transmission of Epstein–Barr virus (EBV) is a very encouraging strategy, especially in haplo-identical transplantation settings [23].

Other specific procedures evaluated by clinical trials include depletion of activated and alloreactive T-cells (i.e. those that can be identified by the expression of the CD25 T-cell receptor subunit). Removal of CD25 T-cells can be done using immuno-selection devices similar to those routinely used for $CD34^+$ positive cell selection or T-cell depletion. Accurate determination of the residual T- and B-cell content is critical. The highest acceptable dose of residual alloreactive T-cells

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must be defined in advance by the medical team in charge of the recipient and their guidance sought by the procurement team if this objective cannot be met.

22.4.5.2. Tumour cell depletion in the autologous setting

Autologous tumour cells procured with normal HPC may contribute to post-transplant relapse, but this has not been firmly established on the basis of clinical and biological observations. A definitive advantage for tumour-purging of autologous grafts has not been demonstrated by clinical trials. The use of $CD34^+$ cell-selection devices for this purpose is only applicable in a few clinical protocols (e.g. neuroblastoma) but, if a transplant team decides to use such a procedure, then detection of residual tumour cells should be as accurate as possible, using either immuno-histochemical techniques or flow cytometry analysis, or molecular biology techniques.

22.5. Quality control

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22.5.1. Biological information needed to confirm donor suitability and recruitment

All clinical and biological information pertaining to donor identification, screening and recruitment must be kept, along with all information pertaining to processing and distribution. This information must remain as a permanent part of the preparation and release file.

Details on the nature of such information and the procedure to obtain it are provided in Chapters 3 and 4 of this Guide.

22.5.2. Safety controls

Detection of transmissible infections is undertaken through donor screening (using micro-biological and other testing, as required by national, European and international guidance and regulations) and through microbiological testing of samples obtained at the different stages of cell procurement, processing and distribution (see also Chapter 5, 8 and 10).

Detection of donor-transmissible diseases other than occult pre-neoplastic or neoplastic diseases or other disorders is through donor screening, using medical questionnaires, physical examination and biological testing, as necessary.

The proportion of the various subpopulations of leukocytes in the procured cell preparations must be measured. High numbers of mature cells such as granulocytes or contamination with red cells may negatively affect several subsequent processing steps and may contribute to recipient side-effects at re-infusion.

Removal of red blood cells through specific processing procedures must be documented, as must the removal of T-cells or other immune effectors.

The removal of tumour cells from autologous cell preparations using specific processing procedures must also be documented where applicable.

22.5.3. Immunophenotyping and colony-forming unit assay

The number of total nucleated cells (TNC) in combination with the number of viable $CD34^+$ cells is a widely used measure for evaluating the quality of procured bone marrow. The cell dose for recipients is usually expressed in TNC and $CD34^+$/kg of recipient weight. In addition, nucleated cell counts are largely used as in-process controls to document that technical procedures have been appropriately conducted in the processing facilities (i.e. procurement of TNC following plasma removal, volume reduction, red blood cell depletion, etc.).

$CD34^+$ cell counts are used as a marker for HPC, both in the peripheral blood of individuals undergoing mobilisation regimes and in the procured cells, whether from apheresis following mobilisation or from bone marrow donation. $CD34^+$ cell counts are usually measured by flow cytometry, using monoclonal antibodies that recognise one or several epitopes on the human CD34 membrane antigen. Use of a single platform, rather than a dual platform, minimises errors in calculating cell counts.

11306 The International Society for Hemotherapy and Graft Engineering (International Society for Cellular
11307 Therapy) algorithm provides a robust and reproducible gating strategy to measure CD34$^+$ cells [24, 25].
11308 Evaluation of CD34$^+$cell recovery and total viability after storage and cryopreservation are an
11309 acceptable way to measure the potency of an HPC graft when the detection of colony-forming units
11310 (CFU) in clonogenic assays is not feasible. These functional tests are hampered by the delay required to
11311 produce results (usually two weeks); thus, the results are usually only available long after a non-
11312 cryopreserved cell preparation has been transplanted in an allogeneic recipient. Clonogenic assays are
11313 also hampered by poor intra- and inter-laboratory reproducibility. This particular issue could be
11314 improved by using commercially available and standardised culture media and by participation in
11315 proficiency testing and external quality-assessment schemes. The frequency of CD34$^+$ cells that form
11316 colonies differs among the sources of HPC and is higher in HPC(A) than in HPC(M). A clonogenic
11317 assay can provide additional information about the functionality of the graft; in particular, it is
11318 recommended after a long storage period. It can be used as a qualitative potency test (e.g. growth or no
11319 growth) or as a quantitative potency test. In both cases, a policy should be defined to deal with grafts
11320 where CD34$^+$ cells clone at a low frequency.
11321 Colonies are enumerated and classified on the basis of their morphologic characteristics: CFU-
11322 GM (granulocytes and macrophages), CFU-GEMM (granulocyte-, erythrocyte-, macrophage-,
11323 megakaryocyte) and BFU-E (burst-forming units – erythroblast).

11324 **22.5.4. Release criteria**
11325 The cell-processing facility, along with its clinical counterparts, must define which safety and
11326 quality controls serve as release criteria. It must also define which criteria must be strictly met and which
11327 ones may lead to documented waivers. Specific instructions should be established in the tissue
11328 establishment on how to deal with the recipient, donor and stem cells throughout the donation, through
11329 the processing and issue stages and all the way through to transplantation. Acceptance and release
11330 criteria may differ between autologous and allogeneic grafts. In autologous grafts, infectious disease-
11331 marker (IDM) test results may be positive (this is normally not the case in allogeneic grafts). In
11332 allogeneic grafts, which are mainly used directly after procurement, microbiological test results are
11333 pending at the time of administration and cannot serve as release criteria (in contrast to autologous
11334 grafts).
11335 Processing and transplant facilities should agree on the cell dose (nucleated cell count,
11336 mononuclear cell count, CD34$^+$ cell count and/or clonogenic assays as appropriate for the source of
11337 HPC) required to achieve reliable and sustainable engraftment.
11338 If cells are required for administration to a patient, a prescription for infusion is required. This
11339 prescription should list the type of cell preparation that is suitable for that patient and provide specific
11340 information on dosing. If necessary, the cells may be manipulated before infusion (e.g. washing,
11341 dilution) and this should be recorded on a worksheet and on the activity report.

11342 **22.5.5. Quality control for MNC**
11343 In addition to the safety controls (listed in 22.5.2) and definition of release criteria (22.5.4), the
11344 specific requirements include establishing the absolute number and the frequency of T-cells (CD3$^+$
11345 and/or subpopulations) and cellular viability by flow cytometry analysis. The anti-tumour effect should
11346 be evaluated at intervals as defined in the clinical protocol.

11347 22.6. Labelling and packaging

11348 Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect
11349 personnel and the environment. The primary packaging must be made of a biologically compatible
11350 material. Cryopreservation requires the use of low-temperature-resistant packaging, which can also
11351 withstand contact with liquid nitrogen.

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Labelling must unambiguously identify the donor, the intended recipient, the cell preparation and its nature, the additives used and the conditions under which the cells are to be stored and distributed. Following procurement, the donor identifier should be always on the 'transit' label when cells are delivered to the processing facility. The recipient must be identified (but not the donor) when cells are distributed for administration. In all cases there must be an audit trail to the donor.

International standards for labelling now exist (e.g. ISBT 128, Eurocode-IBLS) and must be used to promote consistency and traceability, aid international exchanges and facilitate vigilance and surveillance. For tissues and cells procured and distributed in the EU, the Single European Code for Tissue and Cells (SEC) must be used. See also Chapter 14.

22.7. Storage

Storage must be done in conditions that minimise the risk of contamination, cross-contamination and mix-up. A process for quarantine storage should be in place to avoid the possibility that grafts with incomplete or positive IDM test results are accidentally released without proper authorisation.

Conditions for temporary storage must be defined for each type of cell preparation and for each stage of the process, from procurement to release for administration (including pre-processing and post-thawing, etc.). There should be a stability protocol that evaluates the viability and potency of cryopreserved cellular therapy grafts, on a regular basis.

The cryogenic system used for long-term storage must be continuously monitored, and processes must be in place to detect failures in the system, such as temperature rises and changes in the level of liquid nitrogen. An emergency plan should be in place describing the actions to be taken in case a storage device fails, and a procedure should specify how to maintain the cryopreserved grafts at the defined storage temperature.

22.8. Distribution and transport conditions

Internal and external transport must be controlled. Transportation within the same institution (e.g. from the procurement facility to the processing facility, or from the processing facility to the transplant ward) must be defined by SOPs. Periodic container validation and courier qualification should be performed. When service providers are used for transport or shipment of unprocessed or cryopreserved cell preparations, the conditions by which the service is delivered must be established and regularly audited by the cell-processing facility, which remains responsible for the delivery of cell preparations. Appropriate training of the personnel in charge of transportation should be documented. The transport containers should conform to the applicable regulations and should be secured where applicable. For cryopreserved cellular therapy grafts, a dry-shipper should be used. During shipment of HPC grafts, the temperature should be monitored, and records must be maintained by the shipping facility and shared with the receiving facility. See also Chapter 11.

22.9. Biovigilance

As an effective vigilance and surveillance (V&S) system for tissues and cells used in transplantation and assisted reproduction, the EU project SoHO V&S was developed in 2013. The *Guidelines on vigilance and surveillance of human tissues and cells* [26] were published for healthcare professionals responsible for all types of HPC (bone marrow, peripheral blood stem cells, cord blood) for human application. In EU member states, the requirements for traceability, notification of serious adverse reactions and events, and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells are detailed in Directive 2006/86/EC.

Tissue establishments and healthcare professionals should use SoHO V&S guidelines because they provide essential information for the detection, reporting/confirmation and investigation of serious

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adverse reactions and events (SAREs). There is general guidance on implementation of good V&S practice, as well as definitions of SAREs, in Chapter 16.

Tissue establishments should have written procedures for managing SAREs. They should also provide centres carrying out clinical applications with accurate and updated information on various SAREs in the area of HPC transplantation. Moreover, WMDA has set up a central global reporting system for its member organisations to report serious events and adverse reactions related to HPC grafts: Serious (Product) Event and Adverse Reactions or S(P)EAR. The scope of this system is to collect information on serious events and adverse reactions that occurred during procurement or processing of HPC from unrelated donors.

Appendix 29 shows a document of the WMDA listing S(P)EAR examples of what to report and what not to report.

22.9.1. Serious adverse reactions in the recipient

22.9.1.1. Complications related to haematopoietic stem cell infusion

Infusion of HPC is, in general, well tolerated. Complications are consequences of immunological incompatibility, iatrogenic toxicities, microbiological contamination and manufacturing/administrative errors. Some complications are similar to the complications caused by transfusion of standard blood products, which is particularly true for allogeneic HPC preparations if they contain ABO-incompatible RBC or plasma. Other complications are specific to HPC infusion, and are related to allogeneic and autologous HPC.

Adverse reactions can be immunological and non-immunological, acute and delayed.

22.9.1.1.1. Haemolysis of red blood cells

Donor–recipient mismatching in erythrocyte antigens is not a contraindication for HPC transplantation, but haemolytic reactions due to ABO and non-ABO antibodies may occur. Reactions can be acute and delayed. The risk of haemolysis is also dependent upon the type of HPC preparation because the content of RBC and plasma is different.

Acute haemolytic reactions are severe complications of HPC infusion. They are caused by ABO incompatibility between the donor and recipient. Usually, the risk of acute haemolysis is greater if the RBCs of the donor are incompatible with the recipient's plasma (major ABO incompatibility). However, haemolysis can also occur if the donor's plasma is incompatible with the recipient's RBC (minor ABO incompatibility). In general, low titres < 1:64 are associated with mild or no reactions, whereas high titres (e.g. 1:1024) are associated with acute haemolytic reactions. The volume of incompatible RBC infused also determines reaction severity. Bone marrow contains a high amount of RBC and can cause acute haemolysis. The risk of haemolysis can be reduced by removal of antibodies from the patient's circulation or by removal of RBC from the bone-marrow preparations of the donor. In contrast, apheresis preparations usually have < 10–15 mL RBC, which is not enough for significant reactions.

Signs and symptoms of acute haemolytic reactions can be: chills, fever, dyspnoea, chest pain, back pain, headache, hypotension, oliguria, anuria, bleeding, shock and pain along veins. If an acute haemolytic reaction is suspected, cardiovascular and renal function must be maintained and development of disseminated intravascular coagulation prevented.

Delayed haemolytic reactions may occur if the recipient is allo-immunised on the infused RBC antigens of the donor, or if the recipient receives the donor's B lymphocytes within an HPC preparation, which can produce antibodies against the recipient's RBC ('passenger lymphocytes syndrome'). If the recipient is allo-immunised on the donor's RBC antigens, infusion of RBC can stimulate an anamnestic immune response of the residual B lymphocytes of the recipient. The antibody will reach a clinically significant level within 2-14 days after HPC infusion, whereas the infused RBC will remain in circulation. The haemolysis is limited by the amount of infused RBC.

If the recipient receives the donor's B lymphocytes within a HPC preparation that can produce antibodies against the recipient's ABO or other RBC antigens, haemolysis can be more serious because

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of passenger lymphocyte syndrome. At greater risk are recipients who receive ABO minor-incompatible HPC. Typically, haemolysis will occur within 1-3 weeks after HPC infusion. Occasionally, life-threatening haemolysis can occur. Apheresis HPC preparations contain higher numbers of lymphocytes and carry a greater risk of delayed haemolysis.

Signs and symptoms of delayed haemolytic reactions are the same as for acute haemolytic reactions, but the severity differs.

In autologous settings and if cryopreserved HPC are used, passive haemolysis is more common.

22.9.1.1.2. Complications within the respiratory tract

HPC infusion frequently induces complications within the respiratory tract. During administration, patients often start coughing. Coughing is related primarily to application of cryopreserved autologous HPC, and is usually accompanied by nausea and vomiting. Dyspnoea, with mild decreases in the vital capacity of the lungs, is noted quite often.

Severe respiratory complications, such as acute respiratory failure, are rare. Transfusion-related acute lung injury (TRALI) can occur if granulocyte activation in the pulmonary vasculature is caused by neutrophil antibodies or bioactive mediators, which increase microcirculation permeability and allow massive leakage of fluids and proteins into the alveolar space and interstitium. Signs and symptoms of TRALI usually occur < 6 h after HPC infusion, and include acute respiratory distress, low-grade fever, hypoxaemia (oxygen saturation < 90 % on room air) and bilateral pulmonary infiltrates on frontal radiographs of the chest. If hypoxia is observed during HPC infusion, the infusion should be stopped immediately. Respiratory support should be as intensive as dictated by the clinical picture. Supplementation is necessary in almost all cases. Corticosteroids and diuretic drugs are not useful. In severe cases, transfer to an intensive care unit (ICU) may be necessary.

22.9.1.1.3. Febrile non-haemolytic reactions

During HPC infusion, patients may experience febrile non-haemolytic transfusion reactions (FNHTR). These reactions may be observed in allogeneic and autologous transplantation. FNHTR are manifested by a low-grade fever during, shortly after or ≤ 2 h after infusion of cells. FNHTR can be accompanied by chills, rigor and mild dyspnoea without evidence of haemolysis. This phenomenon may reflect the action of antibodies against leukocytes or the action of cytokines (present in infused preparations or generated by the recipient) after cell infusion.

No laboratory tests are helpful in predicting and preventing FNHTR. Any patient with fever, rigor and chills during HPC infusion should be evaluated, clinically and by laboratory tests, for haemolytic, septic or TRALI reactions. An underlying infection must also be excluded. FNHTR are short-lived complications, and anti-pyretic agents usually provide effective symptomatic relief.

22.9.1.1.4. Dimethyl sulphoxide toxicity

Dimethyl sulphoxide (DMSO) is the most widely used cryoprotectant, but it can detrimentally affect cell viability and is the cause of many side-effects observed during infusion. DMSO toxicity is the most common complication of infusion of cryopreserved HPC.

Within minutes of starting the infusion, a metabolite of DMSO is excreted through the lungs and causes a garlic-like odour that can lead to a foul taste in the mouth. Infusion of DMSO can induce a wide range of other symptoms: pruritus; sedation; headache; nausea; vomiting; abdominal cramps; diarrhoea; flushing; low-grade fever; chills; dizziness; garlic-like odour; haemoglobinaemia with red-coloured urine; elevation of levels of hepatic enzymes; elevation of levels of creatinine kinase. DMSO toxicity has been linked to cardiovascular side-effects such as bradycardia or tachycardia, hypotension and, in rare cases, myocardial infarction.

DMSO toxicity is dose-dependent. The maximum daily intravenous dose of DMSO is 1 g/kg, which is equivalent to an infusion of 10 mL/kg of cells in 10 % DMSO solution. Premedication with anti-histamines, slowing the infusion rate, increasing the resting time between multiple infusion aliquots, dilution of thawed HPC preparations by albumin–dextran-40 solution at a ratio of 1:2 or 1:3, or removal of DMSO by washing can prevent symptoms and reduce the risk of DMSO-related toxic effects.

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11493 22.9.1.1.5. Neurological complications
11494 Neurological symptoms during HPC infusion vary widely. Headache is common and can be
11495 related to increased intravascular volume. Occasionally, patients experience more severe side-effects
11496 such as muscle spasms and seizures. Cerebral infarcts and acute encephalopathy are rare. Simple muscle
11497 spasms often resolve spontaneously. For patients with acute mental changes, loss of consciousness or
11498 seizures, urgent intervention is necessary. HPC infusions should be stopped; rapid neurological
11499 assessment should be done as well as basic laboratory tests, including electrolytes and glucose. If
11500 seizures persist, anti-epileptic drugs are indicated. Patients should be transferred to an ICU.
11501 Neurological complications are probably linked to a large number of non-mononuclear cells
11502 and/or caused by DMSO, but this suspicion has not been clearly demonstrated.

11503 22.9.1.1.6. Cardiac toxicity
11504 Cardiac toxicity is common and manifests as bradycardia and other disorders of cardiac rhythm.
11505 It is usually mild (though severe cases of arrhythmias have been reported). Severe bradycardia occurs
11506 more often in recipients of cryopreserved bone marrow, and may require aggressive supportive care.
11507 Aetiology may be because of hypervolemia due to extensive hydration before infusion, large
11508 volume of transplant, hyperosmolality of DMSO, hypothermia, lysis of graft cells or underlying cardiac
11509 conditions.

11510 22.9.1.1.7. Allergic reactions
11511 Allergic reactions usually manifest as urticaria and pruritis. Most occur in patients receiving
11512 allogeneic transplants. Anaphylactic-type reactions are rare. Allergic reactions present as bronchospasm
11513 and/or laryngospasm, hypotension, severe dyspnoea, pulmonary and/or laryngeal oedema, facial
11514 burning and flushing, abdominal pain, diaphoresis, diarrhoea and dizziness.
11515 Causes of allergic reactions are not clear. They may be related to the substances used during cell
11516 procurement, cell processing or cryopreservation, such as HES or DMSO, that can react with antibodies
11517 in the donor or recipient plasma, or with anti-immunoglobulin (Ig)A antibodies in IgA-deficient
11518 recipients. No laboratory tests can help to predict or prevent allergic reactions. Therapy is dependent
11519 upon symptoms. For mild reactions, administration of anti-histamines will be helpful or, in severe cases,
11520 corticosteroids, epinephrine and cardiorespiratory support.

11521 22.9.1.1.8. Anticoagulation effects
11522 Patients receiving non-cryopreserved and non-manipulated bone marrow have a greater risk of
11523 haemorrhage because of high concentrations of unfractionated heparin in bone-marrow grafts.
11524 Risk of bleeding is increased in thrombocytopaenic recipients.

11525 22.9.1.1.9. Hypertension/hypotension
11526 Hypertension is more common in cryopreserved HPC or unmanipulated bone marrow HPC,
11527 because such grafts have a higher volume. Hypertension is a result of acute volume overload due to
11528 rapid infusion, prophylactic hydration and the hyperosmolality of the infused preparation.
11529 Hypotension is also more common in cryopreserved HPC. It is linked with vasodilatation due to
11530 histamine generation. Premedication by anti-histamines decreases the incidence and severity of
11531 hypotension.

11532 22.9.1.1.10. Acute renal failure
11533 Acute renal failure is more common in application of a high volume of cryopreserved HPC. It is
11534 caused by a large amount of DMSO and cellular debris.

11535 22.9.1.1.11. Bacterial contamination
11536 Bacterial contamination of an HPC product is possible. Bone marrow, which is procured into an
11537 open system, has a higher rate of contamination than HPC collecting from peripheral blood.
11538 Contamination may occur at several steps in the process. It can be due to occult asymptomatic
11539 bacteraemia in the donor. For autologous donation, because of the particular nature of the graft and the
11540 recipient's condition due to the treatment, it is vital that HPC are procured irrespective of the possible

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febrile status of the patient, even if sepsis may be present. Although it is a rare occurrence, it should be kept in mind as a potential cause of bacterial contamination of grafts.

Contamination of HPC can also occur during procurement, processing, storage, thawing or sampling, due to an interruption of sterile methods.

After transfusion of contaminated HPC, symptoms of a septic reaction usually develop rapidly. High fever, tachycardia and hypotension, nausea and vomiting, and a 'shock-like' clinical picture should arouse suspicion of bacterial septicaemia. If such symptoms occur during HPC administration, the infusion should be stopped immediately and all infusion bags and equipment examined.

Known bacterial contamination of an HPC unit is not an absolute contraindication for HPC infusion. Patients receiving culture-positive preparations require antibiotic therapy, which can be antibiogram-specific or cover a broad spectrum of bacteria, starting optimally 2 days before transplantation.

There must be criteria for administration of preparations with positive microbial culture results. A contingency plan is expected to be in place in case of urgent medical need.

22.9.1.1.12. Transmission of infectious and genetic diseases

HPC preparations should be tested for transfusion of transmissible diseases according to national requirements. However, the potential risk of transmission of infectious agents by infected donors or cross-contamination during storage cannot be removed completely. A viral infection < 6 months after transplantation must be suspected to be due to transmission of hepatitis B or C virus (HBV, HCV) or human immunodeficiency virus (HIV). For other viral infections the period will differ, depending on the incubation period.

Screening of HPC volunteer donors for genetic disease is mainly based on their medical history and on the results of laboratory tests. The risk of transmission of a genetic disease is higher with cord blood than with the other sources of HPC (bone marrow and PBSC), since some diseases might not be evident at birth or even some months later.

According to the Notify Library, very few cases of genetic diseases transmission have been described after bone-marrow transplants (cyclic neutropaenia, Gaucher's disease). Autoimmune diseases transmission has also been reported (thyroiditis, type 1 diabetes, myasthenia gravis, vitiligo, etc.) [15]. All cases of suspected post-transplantation infection or genetic disease transmission related to HPC infusion must be reported immediately to the procurement site and/or donor registry, who have to follow the requirements of the national vigilance system.

22.9.1.1.13. Engraftment failure

After HPC transplantation, recovery must occur in populations of myeloid, erythroid and immune cells. The earliest sign of haematopoietic recovery is an increase in numbers of granulocytes and platelets in peripheral blood within days and weeks after graft infusion. Engraftment of erythroid lines and immune reconstruction occurs within weeks or months. Engraftment is dependent on the dose and source of progenitor cells, method of cell preparation, function of bone-marrow stroma, intensity of the preparative regimen, donor–recipient relationship and ABO compatibility.

Measurement of granulocyte and platelet engraftments provides essential information about the success of clinical protocols as well as the quality of procurement and processing of HPC.

Primary graft failure for HPC transplantation from bone marrow or peripheral blood is defined as a lack of neutrophil engraftment 28 days after transplantation. Leukocyte recovery is designated as the first of three consecutive days in which the absolute neutrophil count is $> 500 \times 10^6$/L. Platelet engraftment is designated as the first day on which the platelet count is $> 20 \times 109$/L in an untransfused patient. The sign of erythroid recovery is $> 30 \times 109$/L reticulocytes or $> 1\%$ reticulocytes in peripheral blood in an untransfused patient. T-cell engraftment is proof of mixed donor–host chimerism (5-95% donor T-cells). Reasons for failure can be graft composition, graft source, HLA mismatch, ABO incompatibility or other reasons that can be attributed to the patient.

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11589 The transplant unit should report graft failure to the tissue establishment to enable thorough
11590 investigation of the quality and handling of grafts.

11591 22.9.1.1.14. Graft *versus* host disease

11592 GvHD is a serious and potentially lethal complication of allogeneic HPC transplantation. GvHD
11593 occurs if infused T lymphocytes engraft in the recipient and react against the recipient's tissues. Any
11594 allogeneic HPC preparations can cause GvHD. Acute GvHD occurs < 100 days after transplantation,
11595 whereas chronic GvHD occurs > 100 days after transplantation. Risk factors for the development of
11596 GvHD are: donor–recipient relationship (HLA disparity, gender matching, donor parity, donor age, ABO
11597 group mismatching), stem cell graft factors (source and graft composition, cell processing) and
11598 transplantation factors (condition and post-transplant immunosuppression regimens).

11599 Clinical manifestations of GvHD typically involve the skin, liver and gastrointestinal tract in the
11600 acute setting, but can affect (among others) the eyes, oral mucosa, vagina, lungs, joints and neurological
11601 system.

11602 To predict the outcome of acute GvHD, scoring based on organ involvement is important. Chronic
11603 GvHD is more likely in recipients of peripheral HPC than in recipients of bone-marrow HPC. It can be
11604 localised, affect only skin areas, and manifest as progressive systemic sclerosis, Sjögren's syndrome or
11605 primary biliary cirrhosis.

11606 Treatment of GvHD includes high-dose corticosteroids, T-cell-suppression drugs, monoclonal
11607 antibodies targeting T-cells, extracorporeal photopheresis or mesenchymal stem cells (MSC).

11608 **22.9.2. Serious adverse reactions and events related to the graft**

11609 SAREs related to the graft – also referred to here as S(P)EAR (serious product events and adverse
11610 reactions) as defined by the WMDA – can be: inappropriate transportation, receipt of a wrong unit,
11611 receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate
11612 storage in hospital or infusion of a unit into the wrong recipient. The transplantation centre must report
11613 these incidences immediately to the tissue establishment, to the registries if appropriate and, according
11614 to national legislation, to Health Authorities responsible for tissues and cells.

11615 **22.9.3. Serious adverse reactions in haematopoietic progenitor cell donors**

11616 Deaths in unrelated HPC donors are very rare, and few cases have been reported to WMDA. A
11617 small number of deaths have been reported also in related donors, from causes such as subarachnoid
11618 haemorrhage, sickle cell crisis, myocardial infarction and pulmonary embolism. In some of these donors,
11619 pre-existing medical conditions were identified *post mortem*, highlighting the need for stringent medical
11620 suitability criteria and assessment of all HPC donors [11, 12, 26].

11621 Bearing in mind that HPC donations are voluntary and altruistic acts of assumedly perfectly
11622 healthy individuals, it is the ethical and professional obligation of medical professionals and also good
11623 practice to notify, document, investigate and report SARs in the living donor, and not only those
11624 influencing the quality and safety of tissues and cells. SARs are uncommon in healthy donors and rare
11625 types of SAR or emerging trends are likely not to be noticed at the national level. SARs in stem cell
11626 registry donors are followed at the international level by the WMDA. Unfortunately, no consistent
11627 follow-up exists at present for related donors. The European Bone Marrow Transplantation Group
11628 (EBMT) has established a donor follow-up, which is included in the EBMT database [27].

11629 *22.9.3.1. Complications in HPC(M) donors*

11630 Donation of bone marrow is, in general, a safe and well-tolerated procedure, but some mild
11631 symptoms related to induction of general anaesthesia are common. In most cases, donors recover fully
11632 within 2 weeks.

11633 Reactions in bone-marrow donors include constitutional symptoms such as nausea, vomiting,
11634 anorexia, insomnia and fatigue (most common).

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11635 Complications related to puncture of bone marrow, such as pain upon procurement, walking,
11636 sitting and climbing stairs, as well as minor infection, are rare. Bone and soft-tissue trauma at the harvest
11637 site may cause pain, bleeding, oedema or nerve compression. Damage to a lumbosacral nerve root or
11638 penetration into the pelvic cavity or internal iliac vessels may cause severe morbidity. Anaesthesia
11639 carries an unavoidable (albeit very small) risk of life-threatening cardiac or respiratory events, as well
11640 as the possibility of allergic or idiosyncratic reactions to anaesthetic agents. Removal of large volumes
11641 of blood may cause symptoms of hypovolemia or anaemia [15, 17, 20].
11642 Cytopaenias (anaemia, thrombocytopaenia) and more serious reactions such as deep-vein
11643 thrombosis (DVT), thromboembolism, cerebrovascular accident and subdural bleeding have been
11644 documented. Post-donation septicaemia and anaesthesia-related complications have also been described,
11645 as well as respiratory complications such as pulmonary alveolitis and oedema.

11646 *22.9.3.2. Complications in HPC(A) donors*

11647 Complications are related to apheresis and administration of granulocyte-colony stimulating
11648 factor (rhG-CSF). Symptoms related to citrate infusion are the most common.
11649 Additional complications related to apheresis include haematoma, arterial punctures, delayed
11650 bleeding, pain from injury to nerves or tendons, thrombophlebitis, local allergy, generalised allergic
11651 reactions, vasovagal reactions, haemolysis and air embolism. All severe conditions requiring
11652 hospitalisation or intervention, or resulting in death < 24 h after procurement, should be reported
11653 immediately.
11654 Use of rhG-CSF in mobilisation is, in general, safe. Common short-term reactions related to rhG-
11655 CSF are bone pain, headache, myalgia, nausea, vomiting, diarrhoea, fatigue, fever and irritation at
11656 injection site. Most of these effects are reversible after discontinuation of rhG-CSF administration. Other
11657 rare reactions are splenic rupture, anaphylaxis, thrombosis, gout, iritis, keratitis, autoimmune
11658 hyperthyroidism, acute lung injury, capillary leak syndrome, exacerbation of rheumatoid arthritis,
11659 insomnia and reduced numbers of thrombocytes.
11660 Reports from long-term follow-up studies in unrelated and related apheresis HPC donors
11661 mobilised with rhG-CSF demonstrated a similar incidence of leukaemia and other malignancies to those
11662 seen in the general population. All malignant diseases in all donors treated with rhG-CSF should be
11663 reported, regardless of the time of occurrence.

11664 *22.9.3.3. Follow-up of haematopoietic progenitor cell donors*

11665 Chapter 16 on biovigilance also applies to HPC transplantation and must be read in conjunction
11666 with this chapter. The donor, whether related (paediatric and adult donors) or unrelated, should be
11667 followed up by the donation centre in the short, mid and long term according to the policy suggested by
11668 scientific organisations (e.g. Italian Bone Marrow Donor Registry (IBMDR) or EBMT) or as requested
11669 by national regulations (e.g. Austria, Switzerland). In particular, a short-term follow-up to document
11670 SAEs, and a long-term follow-up on a regular basis to document late effects of the donation or the
11671 mobilising agent, should be performed [15, 27].

11672 **22.9.4. Biovigilance of mononuclear cells**

11673 The same requirements as for HPC(A) and HPC(M) apply also to MNC(A) (see above).
11674 The processing of MNC involves several steps where unexpected events that have to be
11675 documented and reported may occur (see §22.9 and Chapter 16), for instance, lower viability of frozen
11676 and thawed MNC than expected or human errors in calculating the dose of T-cells in DLI. In cases of
11677 low viability, DLI may still be used but this has to be documented and a risk analysis carried out. Possible
11678 adverse events associated with DLI are the development of acute and/or chronic GvHD and low blood
11679 counts. These complications may appear after 1 or more weeks after the administration of the $CD3^+$ cells.
11680 As noted in section 22.9, similar complications such as DMSO toxicity, transmission of infectious
11681 diseases or GvHD may occur. In rare circumstances, serious product adverse events/reaction or

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complications in the donor are possible. Follow-up of the donor as stated in section 22.9 and Chapter 16 is recommended.

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Related document:
Appendix 28. Donor search through registries for haematopoietic progenitor cells transplantation
Appendix 29. Examples of what to report and what not to report

Chapter 23. Umbilical cord blood progenitors

23.1. Introduction

Umbilical cord blood (UCB) is a source of haematopoietic progenitor cells (HPC) that can reconstitute the haematopoietic and immune systems. These cells bear unique properties, including a high progenitor cell proliferation ability and immune naïvety. Usually UCB is discarded after delivery, but the blood remaining in the placenta after clamping can be collected, processed and stored safely.

After the first transplant procedure performed in Paris by Professor Gluckman and her team in 1988 in a child with Fanconi anaemia, more than 40 000 patients have been transplanted. According to the World Marrow Donor Association (WMDA), more than 750 000 units are registered at the WMDA Search & Match Service, formerly Bone Marrow Donor Worldwide [1-2]. The database is searchable for any patient in need.

The use of UCB cells offer several advantages, including no risk for the donor, prompt availability as an off-the-shelf medicinal product and clinical benefits like low incidence of graft *versus* host disease, even in partially matched transplants, which increases the patient's chance of finding a suitable donor. UCB banks have therefore facilitated universal access to the therapy, in particular to ethnic minorities. However, there are also some disadvantages: the number of stem cells in UCB is relatively low and often associated to slow engraftment, and it is not possible to use a donor lymphocyte infusion after transplantation. There are interesting approaches to improve outcomes, including the use of very high cellular units, double cord and intrabone transplantation, and promising protocols for progenitor cell *ex vivo* expansion. The most applied protocol in older patients uses reduced intensity conditioning and a double UCB graft approach. Recently, immune active properties like an enhanced graft *versus* leukemia effect have been proposed, and reconstitution of the immune system is the current research area to achieve an improvement of UCB transplant methods [3].

The regulatory framework of UCB collection and processing to make it available to patients in need has evolved considerably over the past two and a half decades. Accreditation and regulation has instilled confidence in clinicians, allowing them to select a UCB unit from across a wide range of banks in many countries.

A UCB bank is a multidisciplinary structure that is responsible for the recruitment and subsequent management of maternal donors as well as the collection, processing, testing, cryopreservation, storage, listing, reservation, release and distribution of units for administration. According to their purpose and organisation, UCB banks can be public, private or hybrid, but all must have a quality system in place to guarantee that the production of UCB units fulfils predefined specifications according to their therapeutic intention. In addition to the legal requirements of Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004, some scientific societies have developed high quality standards. Netcord-FACT and AABB UCB banking standards [4-5] have a worldwide perspective to improve the quality of the products available for transplantation. The accreditation status of the UCB bank is one of the selection criteria for many transplant centres during the search for a UCB unit.

The following generic chapters (Part A) of this Guide all apply to UCB banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, validation and risk management (Chapter 2);
c. Recruitment of potential donors, identification and consent(Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);

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11798 f. Procurement (Chapter 6);
11799 g. Premises (Chapter 7);
11800 h. Processing (Chapter 8);
11801 i. Storage and release (Chapter 9);
11802 j. Principles of microbiological testing (Chapter 10);
11803 k. Distribution and import/export (Chapter 11);
11804 l. Organisations responsible for human application (Chapter 12);
11805 m. Computerised systems (Chapter 13);
11806 n. Coding, labelling and packaging (Chapter 14);
11807 o. Traceability (Chapter 15);
11808 p. Biovigilance (Chapter 16).

11809 This chapter defines the additional specific requirements for UCB banking and transplantation.

11810 23.2. Recruitment of potential donors, identification and consent

11811 23.2.1. Donor recruitment

11812 The therapeutic properties of UCB-derived cells and potentially of their components require the
11813 establishment of a donation programme that enables the safe collection of residual blood contained in
11814 the placenta and cord after appropriate information of the maternal donors.
11815 In general this donation can be made available for public or private uses. Public initiatives
11816 promote donation of UCB unit, usually to a not-for-profit organisation, with the aim to provide UCB
11817 units for transplantation or other approved clinical applications to any patient in need. Many public UCB
11818 banks offer also a service for family UCB banking in case of illness in one of the siblings or other
11819 members of the donor's family [6]. Public UCB banks are generally committed to networking in order
11820 to increase access to UCBs through worldwide registries. In a private bank, the donor keeps ownership
11821 of the product, and the organisation offering the processing and storage services is responsible for
11822 maintenance of the units for future potential medical applications. Due to the nature of these services,
11823 these banks are usually for-profit organisations.
11824 The Council of Europe has been studying the issue of UCB donation for several years and has
11825 always been concerned about the proliferation of UCB banks dedicated to the procurement and storage
11826 of UCB for autologous or family use. This concern resulted in adoption of Recommendation
11827 Rec(2004)8 of the Committee of Ministers to member states on autologous UCB banks, and its
11828 explanatory memorandum [7], which recommends that member states allow establishment of UCB
11829 banks only for altruistic and voluntary donations of UCB. In case of autologous (or family-use) UCB
11830 banks, those organisations must clearly inform parents about the differences between the various
11831 medical objectives of autologous and allogeneic donations and about the uncertainties relating to the
11832 medical applications of autologous UCB preservation. In any case, autologous UCB banks must meet
11833 the same quality and safety standards as for allogeneic UCB donation and banking.
11834 The Council of Europe has produced the brochure 'UCB banking: a guide for parents' to provide
11835 clear, accurate and balanced information about the use of UCB in medical treatment and to guide parents
11836 through their blood-storage options [8]. If a family member with a potential transplantable condition
11837 exists, related UCB units can be collected prospectively and stored for later use.
11838 Donor recruitment (see also Chapter 3) usually starts during pregnancy, with information given
11839 by the woman's healthcare provider, but it may also occur as late as at admission into the maternity unit,
11840 as soon as parents are informed about this possibility and the mother is in good condition for signing an
11841 informed consent.
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Information leaflets or brochures to inform the mothers about CB banking are an important part of the recruitment plan. Information to the donor should include at a minimum:

a. donor exclusion criteria;
b. the potential benefit and risks of UCB donation;
c. testing to be performed;
d. potential use (transplantation or other use);
e. data protection.

Any claims made in recruitment material should be supported by scientific evidence.

It is during pregnancy that expectant mothers are encouraged to gather information and ask questions about the UCB collection procedure. Motivated personnel at collection sites are an important way to approach potential maternal donors. Training of physicians and health professionals on this step is essential to make sure that information provided to parents is accurate and that all their questions can be answered.

23.2.2. Informed consent

Informed consent has to be signed by women who agree to donate UCB of their offspring to a UCB bank. General considerations are shown in Chapter 3. Consent must cover in writing all aspects related to the donation that donors must sign. Normally, there are questions about performing infectious disease marker (IDM) tests, contacting the maternal donor in case an IDM test is positive, using units for research, checking medical notes, etc. Asking for informed consent and providing information about it is not recommended during active labour in order to avoid distraction linked to physical and emotional stress. For private banks, informed consent is usually wider, as it is a contract between parents and bank. In this case all aspects of donation, UCB unit processing and storage, and future uses should be thoroughly explained there. Consent can be obtained in a single step prior to collection, when the donation process is initiated early in the pregnancy, or in two steps: a pre-consent followed by a full consent before the CB unit is placed into a clinical inventory.

23.2.3. Donor evaluation

After maternal donor recruitment, trained personnel must determine the donor's eligibility. It is important to ensure that the donation is safe for future recipients. Maternal and infant donor eligibility must be determined on the basis of the results of screening and testing in accordance with national regulations. To assess donor eligibility, a donor medical history interview, which includes assessment for high-risk behaviours, must be conducted to identify risk factors for transmissible and genetic diseases (see Chapter 4 for further details). The mother will be asked to provide personal and family medical details. There must be written criteria for maternal and infant donor evaluation and management.

In general, transmissible disease, either infectious or genetic, and certain risk behaviours exclude the possibility to donate (see Appendix 31 for more information). The following non-exhaustive list summarises the main risk factors:

a. severe eclampsia;
b. depression, maniac-depressive psychosis not regularly treated, dementia;
c. hepatitis (with few exceptions: see Appendix 31);
d. infertility in treatment or any treatment with growth hormone of human origin;
e. autoimmune diseases;
f. central nervous system diseases, e.g. neurodegenerative diseases, neurofibromatosis, Parkinson, etc;
g. oncological diseases (including neoplastic haematological diseases);
h. infectious diseases (see Appendix 31 for more information).

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11889 In addition to these considerations, there are absolute contraindications that should be taken into
11890 account when evaluating a potential donor [9]; they can be summarised in the following non-exhaustive
11891 list:
11892 a. autoimmune diseases if the mother received treatment in the last 12 months;
11893 b. malignancy (except basal cell carcinoma and in situ cancer treated and cured);
11894 c. inflamatory bowel disease (e.g. Crohn's disease and ulcerative colitis);
11895 d. if the mother has received donated eggs or embryos since 1980;
11896 e. evidence of active or chronic infection;
11897 f. live immunisation (vaccination) during this pregnancy;
11898 g. myasthenia gravis;
11899 h. myelodysplastic or myeloproliferative syndrome;
11900 i. unexplained night sweats;
11901 j. animal bite;
11902 k. organ recipient.

11903 Results of this evaluation must be documented in the clinical history and reviewed by trained
11904 personnel.
11905 The medical history evaluation must be obtained while the mother is able to concentrate on the
11906 answers to the questionnaire and is not distracted by aspects of labour (see a model medical
11907 questionnaire in Appendix 30). The language used must be understood by her. It is not recommended
11908 that either family or friends serve as interpreters or translators. Confidentiality must be preserved. If
11909 responses generate medical concerns, the collection should be rejected or cancelled. The mother's travel
11910 history to endemic areas must be obtained and documented, and eligibility determined according to
11911 national regulations. Screening for human transmissible spongiform encephalopathy, including
11912 Creutzfeldt–Jakob disease, must be documented. If history of communicable disease risk was obtained
11913 in advance of the maternal donor's presentation for delivery, the history must be updated to include
11914 information up to the time of delivery. In the case of a woman who gives birth to an infant donor not
11915 genetically hers, her communicable disease risk history must be obtained. The questionnaire must
11916 include questions to obtain at a minimum genetic history, malignant disease and inherited disorders that
11917 may be transmissible to the recipient.
11918 In addition, IDM tests on maternal blood samples must be performed within seven days before or
11919 after collection of the UCB unit. These samples will be tested for evidence of infection of HIV1, HIV2,
11920 hepatitis B, hepatitis C, syphilis and any additional markers according to local regulations. Assays used
11921 for testing must be validated for use in volunteer blood or tissue donations. According to the EU
11922 regulation, if initial IDM marker tests do not include nucleic acid tests for HIV, HBV and HCV, then a
11923 second IDM test 180 days after donation must be performed. (See Chapter 5 for further details.)

11924 23.3. Procurement

11925 23.3.1. Procurement procedures

11926 Procurement typically involves the following steps:
11927 a. The umbilical cord is clamped as distal from the placenta as possible. No interference with
11928 labour and delivery must occur in order to protect mother and newborn safety. Nowadays, many
11929 obstetrical medical associations recommend delayed clamping. Evidence suggests that an
11930 acceptable time of 1 minute is compatible with public UCB banking [10];
11931 b. A section of the cord is cleaned with a suitable disinfectant;
11932 c. A needle that is attached to the collection bag is inserted into the umbilical cord vein;
11933 d. The collection bag is filled by gravity until the cord looks 'white' and all the blood from the
11934 placenta and umbilical cord is drained into the bag;

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11935 e. The collection bag must be appropriately labelled.

11936 There are two main techniques to collect blood from the cord vein: before the placenta is delivered
11937 (*in utero*) or after the placenta is delivered (*ex utero*). Both procurement techniques give similar results
11938 and their use will depend on the ability of the maternity unit to collect the UCB.
11939 In any case, the individuals performing the collection must be adequately trained. In both cases
11940 the collection bag must contain an adequate volume of anti-coagulant (i.e. CPD) to prevent clotting.
11941 After procurement, the healthcare provider in charge completes a report describing the labour
11942 phase, listing the events to be evaluated for acceptance of the unit, such as presence of fever,
11943 complications, type of delivery, etc. See Appendix 30 for further guidance on how collect this data.
11944 Within the biovigilance process, severe adverse events and reactions need to be notified to the
11945 Health Authority in accordance with established laws and regulations (see also Chapter 16).

11946 23.3.2. Temporary storage and transportation to the tissue establishment

11947 Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, UCB should
11948 be temporary stored in non-frozen conditions after collection, during transportation to the processing
11949 facility. Cell viability decreases and the risk of bacterial growth increases during storage at room
11950 temperature as well as in refrigerators. The distance from the collection and the processing facilities may
11951 be considerable and therefore, the maximum time between collection and the start of processing should
11952 be established. It is recommended that cryopreservation of unrelated UCB units should be initiated
11953 within 48 h while for related units within 72 h. The UCB bank should validate the storage and transport
11954 conditions of the UCB units.
11955 The UCB procurement bag will be identified with the following labels and paperwork:
11956 a. unique UCB and maternal codes;
11957 b. product name;
11958 c. procurement site name or identifier;
11959 d. date/time of collection;
11960 e. name and volume/concentration of anticoagulants;
11961 f. recommended storage temperature;
11962 g. biohazard sign and/or other warning labels, following national regulations;
11963 h. statement 'Related donor' where applicable;
11964 i. donor name for related UCB units;
11965 j. recipient family or individual name if known.

11966 Transportation should be done using the same criteria as other progenitor cell products, especially
11967 in the requirements for containers, temperature monitoring and labelling (see Chapter 11). Shipping
11968 method must be validated and meet transportation regulations for this type of product. Upon receipt, the
11969 integrity of the UCB units and their containers will be checked, and any deviation will be recorded
11970 within the processing records.

11971 23.4. Processing of umbilical cord blood progenitor cells

11972 A UCB bank must have appropriate facilities and personnel for the reception, processing, testing and
11973 storage of UCB and maternal blood. All processes should be performed in compliance with national and
11974 EU regulations. Where aspects of processing, testing or storage are performed by an external party, there
11975 must be a written agreement in place between the UCB bank and the external party providing the service.
11976 Factors influencing the air-quality specification for processing HPC from umbilical cord also apply (see
11977 Table 22.5 and Chapter 7).

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11978 A UCB bank structure needs to co-ordinate different lab facilities, including a processing
11979 laboratory, a cryogenic storage area and associated testing laboratories for quality control of individual
11980 batches.

11981 **Table 23.1. Structural components of an umbilical cord blood bank**

Facility	*Characteristics*
Procurement	Collection must take place in a maternity unit and be performed by trained health professionals using a validated technique without any interference in labour care. Besides the general requirements described in Chapter 6, a collection site must ensure secure storage of the CB unit, associated samples, maternal samples and related documentation until they are transported to the CB Processing facility
Processing	All general requirements described in Chapter 8 apply. Processing must be secure and have adequate space to perform all activities in a safe and sanitary manner. Relevant environmental conditions that could affect the safety and potency of the CB unit need to be defined and monitored
Cryostorage	UCB units must be stored in either liquid or vapour-phase liquid nitrogen below −140 °C. All refrigerators, freezers and cryostorage tanks used for storage of UCB units, associated reference samples, and maternal samples, must have a system to continuously monitor and regularly record the temperature. There must be an alarm system in place 24 h a day. Additional storage devices of appropriate temperature must be available in the event that a primary storage device fails
Testing	Agreements must be in place with laboratories performing cell counts, flow cytometry and potency assay. Other laboratories needed are: • accredited human leukocyte antigen (HLA) laboratory, • immuno-haematology lab, • a certified laboratory for IDM testing, • laboratories for haemoglobin screening. Testing should be undertaken in accordance with national and international regulations (see also Chapter 5).
Administrative area	UCB banks need to communicate with maternal donors, registries and transplant centres. A quality assurance system must ensure that CB units become available for search on a registry only after processing, medical and quality review has been completed. Documentation related to request for CB unit or for samples, results of testing and records and transportation and shipping between facilities .must be retained in accordance with applicable national laws and regulations

11982
11983 Setting up a high-level processing lab is very important since there is a clear correlation between
11984 good practice, quality certification and the outcome of UCB transplantation [11].

11985 **23.4.1. Reception at processing facility**

11986 On receipt of a UCB unit, a series of checks needs to be performed on the unit, on the associated
11987 samples and on the accompanying documentation, to verify and determine whether specific acceptance
11988 criteria are met. These include parameters such as volume, total nucleated cell (TNC) content, correct
11989 documentation and labelling, signed maternal donor consent, appropriate transport temperature, absence
11990 of large/multiple clots, transport conditions and acceptable time in transit from procurement centre to

11991 processing laboratory. Once a UCB unit meets the initial acceptance criteria it will continue on to be
11992 processed.

11993 23.4.2. Volume/red blood cell reduction

11994 Despite some loss of cells, volume reduction including red blood cell (RBC) depletion has
11995 practical and clinical benefits: it allows efficient storage of UCB in terms of space and cost, and, most
11996 importantly, it eliminates the following when infused after thawing:

11997 - potentially ABO-incompatible plasma,
11998 - free haemoglobin,
11999 - RBC stroma.

12000 The eliminated RBC and plasma components can be used for immediate or future testing, thereby
12001 minimising the loss of the actual UCB product for testing purposes [12].
12002 The final product volume and cellular characteristics are dependent on the starting product as well
12003 as the processing/separation technique. Over the past decade three major methods have been used in
12004 large-scale banking which produce reproducible results that could be standardised. These include the
12005 manual method using hydroxyethyl starch (HES) for RBC sedimentation or other proprietary reagents,
12006 the semi-automated bottom-and-top method, and newer fully automated and programmable closed
12007 systems.
12008 Whichever platform is employed, it is essential that the equipment and reagents used do not
12009 adversely affect the viability of the cells, that the process does not allow the introduction of adventitious
12010 agents or the transmission of communicable disease, and that the method be validated to allow optimal
12011 recovery of the fraction of interest.

12012 23.4.3. Cryopreservation, thawing and infusion

12013 *23.4.3.1. Cryopreservation*

12014 The selection of a suitable protocol for cryopreservation of UCB is critical to optimise the
12015 recovery of functionally viable HPC [13]. Potential causes of cell damage include type and concentration
12016 of cryoprotectant, cell concentration, and cooling and warming rates, as well as level of control of
12017 storage conditions (see also Chapter 9).
12018 Standard operational procedures (SOPs) related to cryopreservation should specify that the
12019 following information is recorded for each unit:

12020 a. TNC concentration within a defined range;
12021 b. the type of cryoprotectant, its final concentration, and the duration of the cell exposure prior to
12022 freezing;
12023 c. method of freezing and end-point temperature of cooling;
12024 d. cooling rate within a defined range;
12025 e. freezing curve parameter within a defined range;
12026 f. storage temperature.

12027 UCB units must be stored in freezing bags designed and approved for the cryopreservation of
12028 human cells and placed into metal cassettes to provide protection during freezing, storage, transportation
12029 and shipping. It is important that, after filling, each freezing bag is visually examined for possible leaks
12030 and breakage of seals. As reference samples each freezing bag must have integrally attached, at
12031 minimum, two segments of adequate volume to assess identity and potency of the CB cells prior to
12032 release.
12033 UCB units should be cryopreserved using a controlled-rate freezer with a validated freezing
12034 program. The majority of UCB banks use cooling rates of 1-5 °C/min in order to allow the cells to slowly

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12035 dehydrate as the ice phase progresses and the extracellular solute concentration increases.
12036 Cryoprotectants used for UCB are those generally established for other HPC sources. In general, a
12037 concentration of 10 % DMSO is considered optimal for UCB. When used in conjunction with DMSO,
12038 other cryoprotectants, like Dextran-40, enhance the cryoprotective effect by allowing stabilisation of the
12039 cell membrane. While alternatives have been proposed, it is generally considered that a combination of
12040 10 % DMSO and 1 % Dextran-40 results in the best recovery rates for TNC, $CD34^+$ and colony-forming
12041 units (CFU). Prolonged exposure of cells to DMSO can result in damage to cells. It is therefore essential
12042 that the duration from addition of cryoprotectant to initiation of freezing is minimised and the maximum
12043 time allowed should be validated by the bank.
12044 In addition to the two contiguous segments, for each banked unit it is necessary to store several
12045 samples. FACT-Netcord standards require, from each UCB unit, at least 2×10^6 TNC divided in two
12046 vials, suitable material for preparation of genomic DNA, and plasma; and, from the maternal donor,
12047 serum and/or plasma and suitable material for preparation of genomic DNA. All the samples must be
12048 stored at −70 °C or colder. Representative samples intended for viability or potency analysis must be
12049 stored under the same conditions as the CB unit.

12050 *23.4.3.2. Thawing and infusion*

12051 It is important to ensure that the transplant centre receives information on how to handle and use
12052 the UCB unit. Handling includes thawing, dilution and washing of the UCB unit. Providing information
12053 about indications, contraindications and cautions is the responsibility of the UCB bank. A jointly
12054 prepared document, *Circular of information for the use of cellular therapy products*, is available online
12055 [4, 14]. Along with this circular, UCB banks should be able to provide instructions for a validated
12056 thawing method of their UCB unit. Units that have not been red cell reduced prior to cryopreservation
12057 should be washed, as recommended by JACIE, while a buffy coat enriched UCB unit can be simply
12058 diluted [15] see also § 22.4.4.3 .

12059 ## 23.5. Quality control

12060 ### 23.5.1. Biological information needed to confirm donor suitability and recruitment

12061 All clinical and biological information pertaining to donor identification, screening and
12062 recruitment must be kept, along with all information pertaining to processing and distribution. This
12063 information must remain as a permanent part of the preparation and release file, see Chapters 2 and 15
12064 for more details.
12065 Details on the nature of such information and the procedure to obtain it are provided in Chapters
12066 3 and 4 of this Guide.

12067 ### 23.5.2. Safety controls

12068 In order to provide a safe UCB product for release, it is essential that UCB units are screened and
12069 tested for communicable diseases (see also Chapter 5). Maternal blood obtained within 7 days before or
12070 after the collection of the unit is used as a surrogate test for IDMs, and is strongly reflective of the
12071 infectious status of the UCB units due to the shared circulation during gestation. Testing the UCB unit
12072 for IDM provides an additional degree of safety. At a minimum, prior to release for administration, the
12073 maternal donor of each UCB unit must be tested for evidence of infection by at least the following
12074 communicable disease agents:
12075 a. Human immunodeficiency virus, type 1;
12076 b. Human immunodeficiency virus, type 2;
12077 c. Hepatitis B virus;
12078 d. Hepatitis C virus;
12079 e. *Treponema pallidum* (syphilis);

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12080 f. any additional agents required by national regulations or locally endemic disease.

12081 A medical and genetic history of the infant donor's family must also be obtained to prevent the
12082 transmission of malignant diseases and inherited disorders.
12083 UCB units for unrelated use must be shown to be free of microbial contamination. Microbial
12084 testing must be performed using a system validated for the growth of aerobic and anaerobic bacteria and
12085 fungi. For related UCB units, the results of positive microbial tests must include identity and
12086 antibiogram(s) of the organism(s), and these results must be reported to the prospective clinical
12087 programme.
12088 Prior to release for administration, each UCB unit must have undergone haemoglobinopathy
12089 screening, regardless of the family's ethnic background or history.
12090 Mechanisms for donor counselling should be in place if there is a positive test result for any IDM
12091 (other than *Cytomegalovirus*), an abnormal haemoglobinopathy screening or any other abnormal test
12092 finding. Every effort should be made to notify the mother, and/or her physician. The UCB bank must
12093 have policies for handling specific cases.

12094 **23.5.3. Quality specifications**

12095 In order to characterise a UCB unit, identity, purity and potency assays must be performed and
12096 evaluated. Table 23.2 shows a list of reference values suggested by 6th edition of the Netcord–FACT
12097 standards to determine the quality of a UCB unit stored for clinical administration.

12098 **Table 23.2. Specifications of requirements for unrelated umbilical cord blood units**

Test	*Specification*
Total nucleated cell count	$>5 \times 10^8$
Total nucleated cell recovery	Should be >60 %
Fresh TNC viability	>85 %
Viable CD34 counts	$>1.25 \times 10^6$
Fresh CD34 viability	>85 %
Post-thaw CD34 viability	>70 %
Post-thaw CD45 viability	>40 %
Post-thaw CFU	Growth
Sterility	Negative for aerobes, anaerobes and fungi
Donor screening and testing	Compliant with applicable law and regulation
Identity	HLA, ABO verified

Source: modified from 6th edition of Netcord–FACT standards.

12099
12100 Meeting UCB quality specifications and having very good banking practice will ensure a
12101 successful UCB transplantation [16].

12102 **23.5.4. Release criteria**

12103 The UCB bank must receive a formal request from the transplant centre before the work-up starts.
12104 Return of unrelated UCB units is generally not permitted.

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12105 The three tests to be performed by the bank before a UCB unit can leave storage premises are:
12106 verification of donor identity; potency assessment; and safety evaluation. These tests are summed up
12107 below.

12108 *23.5.4.1. Verification of donor identity*

12109 UCB unit identity can be verified by performing HLA-typing using a segment physically attached
12110 to the freezing bag containing cryopreserved UCB cells. The UCB bank must have a policy in place for
12111 the cases where there are no remaining attached segments. Verifying the maternal HLA haplotype would
12112 add additional safety requirements to validate HLA typing and to ensure maternal testing and assessment
12113 corresponds to the product selected [17].

12114 *23.5.4.2. Potency assessment*

12115 It is required to assess the functional capacity of the UCB unit prior to release to the transplant
12116 centre. CFUs are grown from functionally viable cells and the results of this assay increase confidence
12117 in UCB unit quality and ability to engraft. Therefore, it is recommended to perform CFU assay from a
12118 frozen contiguous segment prior to release for administration.

12119 *23.5.4.3. Safety evaluation*

12120 IDM testing of the maternal samples is understood to be a surrogate test, and strongly reflective
12121 of the infectious status of the UCB unit. Prior to release for administration the results of maternal donor
12122 screening must be available. Because of differing national regulations, testing for additional infectious
12123 agents by IDM test may be required by the transplant centres.

12124 23.6. Labelling and packaging

12125 Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect
12126 personnel and the environment. The primary packaging must be sterile and made of a biologically
12127 compatible material. Cryopreservation requires the use of liquid-nitrogen-resistant bags.
12128 From procurement to distribution, labelling must unambiguously identify the UCB unit. Each
12129 label must include at least the unique identifier, the proper name of product, the intended recipient (if
12130 known), the type of manipulation, the anticoagulants and additives used and the conditions under which
12131 the cells are to be stored and distributed. The recipient must be identified (but not the donor) when cells
12132 are distributed for administration. Labelling must allow the UCB bank to ensure the link between the
12133 UCB unit and its samples and records. Because UCB bags are normally too small for a standard-size
12134 label, a partial label at distribution is acceptable and must include at least a unique numeric or
12135 alphanumeric identifier, the proper name of the product and the product code. Additional information
12136 can be included in a tie tag and /or in the accompanying documentation.
12137 International standards for labelling cellular therapy products are now available (e.g. ISBT128,
12138 Eurocode) and their implementation is required by specific accreditation bodies. Global labelling
12139 systems promote consistency and traceability, aid international exchanges and facilitate vigilance and
12140 surveillance. For tissues and cells procured and/or distributed in the EU, the Single European Code
12141 (SEC) must be used. See also Chapter 14 and the EU coding platform [18].

12142 23.7. Storage

12143 In addition to the general requirements described in Chapter 9, the long-term storage required, UCB
12144 banks must have an inventory management system to ensure that each UCB unit and its associated
12145 reference samples, maternal samples and records can be located in a timely manner. This inventory
12146 management system should prevent mix-ups or contamination of the UCB units during storage, and
12147 address the duration of the storage for cryopreserved UCB units. The UCB banks need to establish and
12148 validate the duration and conditions of storage; the effects of long-term storage on the viability, potency
12149 and sterility of the UCB cells should be evaluated in a stability protocol. A procedure for quarantine to
12150 minimise the risk of microbial cross-contamination of UCB units must be in place. Release of a CB unit

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from quarantine should be based upon the evaluation of the testing and screening results pertinent to that UCB unit, in accordance with applicable national laws and regulations.

Refrigerators and freezers used for the storage of UCB units and all associated reference products should not be used for any other purpose, in order to minimise the risk of cross-contamination.

UCB units are intended for long-term storage and must be stored at −140 °C or colder. Each facility should assess the potential risk of transient warming events during processing and/or storage. Examples of these events include transfer of UCB units from the controlled-rate freezer to the cryostorage tank, removal of segments for confirmatory testing and storage of UCB units in vapour vessels that may exhibit unstable temperatures when open. Each step should be validated to show that the viability and potency of the UCB unit have not been compromised.

23.8. Distribution and transport conditions

Internal and external transport or shipping must be controlled, and records must allow tracking and tracing of the UCB unit from UCB bank to the transplant centre. Methods of transportation and shipping must be described in operating procedures. Container validation and courier qualification should be performed periodically. Transport containers must be appropriately labelled and secured, and must conform to applicable regulations. For shipment of the cryopreserved UCB units, a dry-shipper must be used, and the temperature monitored and recorded to detect temperature excursions. A plan for alternative transportation or shipping in an emergency should be in place. Transportation records must be maintained by the shipping facility and shared with the receiving facility. Appropriate training of the personnel in charge of transportation should be documented. See also Chapter 11.

23.9. Biovigilance

Adverse events and reactions (serious and non-serious) must be recorded, reported and investigated according to corresponding national regulations for tissues and cells as described in Chapter 16.

In EU member states, the requirements for traceability, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells are detailed in Directive 2006/86/EC.

Tissue establishments must have standard operational procedures (SOPs) for managing serious adverse reactions and events (SAREs). They should also provide centres carrying out clinical applications with accurate and updated information and training on SAREs in the area of HPC transplantation.

Tissue establishments and healthcare professionals may use EU SoHO V&S guidelines as they provide essential information for the detection, reporting/confirmation and investigation of SAREs. There is general guidance on implementation of good vigilance and surveillance practice, and definitions of SAREs, in Chapter 16.

23.9.1. Serious adverse reactions and events in the recipient

23.9.1.1. Complications related to UCB stem cell infusion

This topic is also discussed in Chapter 22 (see §22.9.1). In the use of UCB the most frequent reactions are those related to effects derived from infusing incompatible RBC, cryoprotectant or other adventitious substances used for volume reduction. The most common infusion reactions are hypertension, nausea and vomiting, bradycardia and chest pain. Table 23.3 summarises the most common reactions.

12193

12194 **Table 23.3. Common reactions after umbilical cord blood infusion**

Immune reactions	haemolysis of red blood cells febrile non-haemolytic reactions allergic reactions
Systemic complications	complications within the respiratory tract neurological complications cardiac toxicity acute renal failure anticoagulation effects
Biological	engraftment failure graft *versus* host disease
Transmissible	bacterial contamination transmission of infectious and genetic diseases

12195

12196 23.9.2. Serious adverse reactions and events related to the product

12197 Despite following JACIE guidelines on UCB, there may occur what are called serious product
12198 events and adverse reactions, such as inappropriate transportation, receipt of a wrong unit, receipt of a
12199 damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in
12200 hospital or infusion of a unit into the wrong recipient. In all such cases the transplantation centre must
12201 immediately report this matter to the tissue establishment and, according to national legislation, to the
12202 Health Authorities. If clinically relevant, it is recommended that a policy to inform the donor is adopted
12203 if there are donor consequences of (genetic) findings in donor cells in the recipient.

12204 23.9.3. Serious adverse reactions and events in umbilical cord blood progenitor cell donors

12205 *23.9.3.1. Follow-up of UCB progenitor cell donors*

12206 The UCB bank must have a policy for the follow-up of both maternal and infant donors and for
12207 the management of donation-associated adverse events. The policy must define the time period within
12208 which to contact donors (see Chapter 2 for futher details).

12209 23.10. References

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12250 **Related documents:**
12251 **Appendix 30: Health assessment questionnaire cord blood donors**
12252 **Appendix 31: Data collection cord blood donor**
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Chapter 24. Pancreatic islets

24.1. Introduction

Type-1 diabetes mellitus (T1DM) is characterised by absolute and specific destruction of insulin-producing cells that reside within clusters of cells in the pancreas known as islets. People who do not have diabetes mellitus have ≈ 1 million islets comprising 2 % of the overall pancreas. Without lifelong insulin replacement, T1DM quickly results in coma and death. Even with optimised treatment, vascular and neurological complications often develop over time. Restoring near-normal blood glucose levels can prevent these complications. This has, however, been associated with a threefold increase in severe hypoglycaemia (low blood glucose, which can result in collapse without warning, one of the greatest fears for those living with insulin injections) [1]. Diabetes mellitus remains a leading cause of blindness, renal failure (requiring dialysis or renal transplantation) and lower limb amputation.

The ultimate goal of pancreatic islet transplantation and beta-cell replacement therapy is to restore glucose-responsive insulin secretory capacity to patients with insulin-deficient DM. This includes all people with T1DM and potentially also those with insulin-deficient type-2 diabetes mellitus (T2DM). The benefits of islet transplantation (in its current form) are reversal of life-threatening hypoglycaemia unawareness, with improved glycaemic control overall and, in over 50 % of patients, complete insulin independence for variable periods of time. It should, therefore, be available for patients who have unresolved recurrent severe hypoglycaemia despite optimised specialist management.

Hence, islet transplantation may be especially beneficial for two defined subgroups of people with T1DM: those patients with severe hypoglycaemia without warning signs and those patients with unstable diabetic control following renal transplantation [2]. Also, islet autotransplantation – as an adjunct to total pancreatectomy for benign pancreatic disease (e.g. for chronic pancreatitis) – can prevent the labiality of surgically induced severe DM.

The following generic chapters (Part A) of this Guide all apply to pancreatic islet transplantation and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, validation and risk management (Chapter 2);
c. Recruitment of potential donors, identification and consent (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);
h. Processing (Chapter 8);
i. Storage and release (Chapter 9);
j. Principles of microbiological testing (Chapter 10);
k. Distribution and import/export (Chapter 11);
l. Organisations responsible for human application (Chapter 12);
m. Computerised systems (Chapter 13);
n. Coding, labelling and packaging (Chapter 14);
o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

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24.2. Donor evaluation

24.2.1. General criteria

Donor criteria for pancreatic islet transplantation are the same as those generally applied for pancreatic transplantation [3]. All suitable deceased donor pancreases that have not been placed for vascularised whole organ transplantation should be allocated for pancreatic islet transplantation according to a prioritised (inter)national waiting list. However, additional criteria for donation of tissues and cells (see Chapters 3 and 4) must be applied.

24.2.2. Donor characteristics

Donor characteristics – such as body surface area, body mass index and number of vasopressor types used – are predictors of successful pancreatic islet isolation. Other characteristics such as age, cold ischaemia time, and blood chemistry levels of glycated haemoglobin A1c, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), blood urea nitrogen, amylase, lipase, sodium and glucose could influence pancreatic islet isolation yield [4-6]. The tissue establishment should establish contraindications for pancreas acceptance.

24.2.3. Specific exclusion criteria for pancreatic islet transplantation

Donors suffering from diabetes mellitus type 1 or 2 are excluded from donation for this clinical use.

24.3. Procurement

The consistency of pancreatic islets manufacturing is highly dependent on the quality of the procured organ. Organ procurement should be conducted to ensure organ viability in transit, using similar procedures as for whole pancreas transplantation, but vascular access is not required. If a distant team has procured donor pancreases, the tissue establishment should have agreements with the procurement centre(s) on organ harvesting, warm and cold ischaemia time, organ preservation methods, cold preservation fluid and shipping conditions.

24.4. Processing and storage

Organs are transported to the designated isolation facility. Pancreases are processed by enzymatic and mechanical dissociation, and pancreatic islets are usually collected after density-gradient purification.

Storage of pancreatic islets in media under stringent conditions before implantation has logistical benefits: it enables additional quality-control tests, and allows time to prepare the patient for transplant or to ship pancreatic islets to a distant transplant centre [7]. The tissue establishment should guarantee that the composition of the storage medium does not alter the physiological properties of insulin-producing islets.

24.5. Quality controls/release criteria

Pancreatic islet cells exhibit a wide variety of functions that should be tested during quality control procedures. The tissue establishment should define – alongside the general tissue-and-cell release criteria – additional criteria for pancreatic islet transplantation, including:

a. quantification of the pancreatic islet cell mass (total islet number and the islet equivalent, known as IEQ), or of the number of insulin-positive cells;
b. cell viability (e.g. qualitative determination by Hoechst/propidium iodide, fluorescein diacetate/ethidium bromide or functional assessments);
c. microbiological testing;

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d. bacterial endotoxin testing [8] (see §10.3.4);
e. beta-cell function (e.g. glucose-stimulated insulin secretion or insulin synthesis); but this information is not available prior to transplantation in all programmes.

Many of the currently utilised biological assays measuring islet functions and sterility are not always rapid enough for use in routine release testing because of the short period between pancreatic islet isolation and transplantation, varying from several hours to a few days. The tissue establishment should define how it will deal with incomplete test results.

Following confirmation of product identity and integrity of the pancreatic islet graft, islets will be transplanted into the portal vein as an inpatient procedure. Alternative routes of administration should demonstrate adequate safety.

24.6. Packaging and distribution

Transport temperature is usually maintained at 12-25 °C.

Pancreatic islet cells are transported in liquid media, so special notice might be necessary, depending on airline transport regulations (see Chapters 11 and 14).

24.7. Traceability

The attached documentation for the clinical transplantation centre should include, for example, details of the donor, organ transport/ischaemic time, pancreas quality, quantification of the pancreatic islet cell mass or of the number of insulin-positive cells, sterility, viability and function. Records covering the complete process from donor to recipient should be kept at the tissue establishment, and it should be possible to trace also other organ recipients from the same donor, and vice versa.

24.8. Biovigilance

Any unforeseen events influencing islet isolation and storage conditions are to be considered as adverse events that should be recorded, and reported to the competent authority. Some examples are:

- loss of pancreatic islets during isolation or temporary storage, due to failure of equipment and monitoring systems (e.g. overheating, carbon dioxide concentration, cooling),
- loss of pancreatic islets due to incorrect use of media (e.g. pH problems, sterility of media, concentration of additives, shelf-life).

Although islet transplantation is a relatively safe procedure, serious adverse reactions are not infrequent (see Table 24.1). Data collected by the Collaborative Islet Transplant Registry (CITR) show that one-third of all islet recipients have experienced at least one serious adverse reaction (SAR) in the first year after islet transplantation. Most of the reported SARs were related to the immuno-suppression therapy and the islet infusion procedure (bleeding and blood clots, intraperitoneal or liver subscapular). Approximately 91 % resolved with no residual effects [9, 10]. The incidence of SARs has declined significantly in recent years. Life-threatening events occurred in 24 % of recipients in 1999-2003 and only 4 % in 2011-2014 [10, 11]. For further guidance on biovigilance, please refer to Chapter 16.

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12373 **Table 24.1. Non-exhaustive list of possible reportable SARs in islet transplant recipients [10, 11].**

Infusion procedure-related
• haemorrhage • portal thrombosis • transient transaminitis
Immuno-suppression-related
Haematological • anaemia • leukopaenia • neutropaenia **Metabolic** • dyslipidemia **Gastro-intestinal** • oral ulcers (sirolimus) • diarrhoea (mycophenolic acid) • CMV colitis **Respiratory tract** • upper respiratory infections • interstitial pneumonitis (sirolimus) **Neurological** • neurotoxicity (tacrolimus) **Genito-urinary** • urinary infections • ovarian cysts • dysmenorrhoea • nephropathy • proteinuria **Cutaneous** • infections • cancer

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12374 24.9. Developing applications for patients

12375 In the last several years, some innovative applications have been investigated and developed, based on
12376 somatic cell and gene therapy:
12377 • human embryonic stem cells differentiated into pancreatic beta-cell precursors [12];
12378 • encapsulation of insulin-producing cells;
12379 • hepatic insulin gene therapy (pre-clinical).

12380 24.10. References

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Chapter 25. Hepatocytes

25.1. Introduction

Hepatocyte transplantation is an alternative treatment to liver transplantation for patients with metabolic liver diseases or acute liver failure, or as a temporary support for patients with liver failure while waiting for an organ transplant [1, 2]. Patients with metabolic liver diseases are characterised by deficiency of one particular enzyme or protein, giving rise to hepatic and/or extrahepatic disease while all other liver functions are unimpaired. Thus, replacement of the whole liver by liver transplantation may not be required, and selective replacement of a fraction of the liver cell mass should be therapeutic. There is evidence that replacement of 5 %-10 % of the liver with healthy donor hepatocytes can correct a wide range of inherited metabolic liver diseases [3, 4]. In patients with acute and chronic liver failure, hepatocyte transplantation could provide temporary liver support until the native liver has recovered or a whole liver is available for transplantation.

Hepatocyte transplantation has potential advantages over whole-organ transplantation: the procedure is a less invasive approach, resulting in lower morbidity and mortality; it can be repeated several times; and it is reversible. Functional hepatocytes can be isolated from unused segments of donor livers that had been retrieved for whole-organ transplantation; and, in contrast to whole organs, cells can be cryopreserved and stored until needed.

This chapter defines the additional specific requirements for liver tissue and hepatocyte isolation and transplantation. The following generic chapters (Part A) of this Guide all apply to hepatocyte isolation and transplantation and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, risk management and validation (Chapter 2);
c. Recruitment of potential donors, identification and consent (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);
h. Processing (Chapter 8);
i. Storage and release (Chapter 9);
j. Principles of microbiological testing (Chapter 10);
k. Distribution and import/export (Chapter 11);
l. Organisations responsible for human application (Chapter 12);
m. Computerised systems (Chapter 13);
n. Coding, labelling and packaging (Chapter 14);
o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

25.2. Donor evaluation

Liver tissue for hepatocyte isolation can be procured from donors after brain death (DBD) and from donors after circulatory death (DCD).

Liver tissue can also be procured from healthy living donors. In theory a healthy living donor could donate a part of their liver. However, this procedure has been rarely performed so far because of the risk of morbidity and mortality for the living donor when balanced against the results obtained in clinical hepatocyte transplantation [5]. The liver from a living donor can also be the explanted liver in a

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so-called domino procedure where a patient is undergoing a liver transplantation [6], provided that the indication for the transplant (for example maple syrup urine disease) [7] is not considered to be a contraindication for the hepatocyte recipient. However, explanted livers of patients with familial amyloidotic polyneuropathy (FAP) are usually used for transplantation in another recipient rather than for the preparation of hepatocytes.

At present, hepatocyte transplantation is limited by the scarcity of donor livers rejected for whole-organ transplantation, that is, by the lack of adequate sources for viable human hepatocytes. Steatotic donor livers, which are becoming more common with the increasing incidence of obesity in European populations, are currently not considered to be a viable source of cells for hepatocyte transplantation, as steatotic hepatocytes display impaired metabolic function and lower engraftment [8]. The average hepatocyte yield after perfusion varies from 3×10^6 to 2×10^7 hepatocytes per gram of tissue, with variable viability yields reported (20-85 %) [8]; several billion cells are generally infused into one patient. Primary human hepatocytes do not proliferate *in vitro* and therefore cannot be expanded. Cryopreservation may have harmful effects on the viability and metabolic function of the cells [9].

All these limiting factors have prompted researchers and clinical teams to investigate the use of hepatocytes produced by the differentiation of pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) [10], which can both be indefinitely amplified and have the potential to become a permanent source of quality-controlled hepatocytes. Clinical-grade hepatocytes derived from these cells are now being produced by small companies and should be tested for clinical use in the coming years.

Donor criteria for hepatocyte donation are the same as those generally applied for organ donation for liver transplantation. Donors positive for HIV, HBV or HCV, as well as for malignant tumours, are excluded. Conditions to be evaluated as part of the donor-selection process are:

a. liver-originated disease of the donor that could be transferred to the recipient and cause disease, e.g. hyperoxalosis, familiar amyloidotic polyneuropathy;
b. alterations to the liver vessels that could complicate perfusion and isolation of hepatocytes (though this is uncommon);
c. donor liver characteristics that might affect hepatocyte quality, such as the size of liver tissue, the degree of steatosis and the length of both warm and cold ischaemia and hypoxia [11, 12].
d. Neonatal livers are not generally considered for organ transplantation in view of the increased incidence of thrombosis and due to size limitations. Neonatal livers may, however, be a valuable source of hepatocytes and their function is comparable to (may even be superior to) hepatocytes derived from adult donors. Post-thaw viability of cryopreserved neonatal hepatocytes is significantly higher when compared to adult hepatocytes [13, 14].

25.3. Procurement

Liver tissue is usually procured from deceased donors by the surgical liver-retrieval team of the transplant unit. Staff performing the procurement must be adequately trained in liver retrieval. Liver tissue should be flushed either *in situ* or *ex vivo* with an appropriate organ-perfusion solution through the hepatic artery and/or portal vein.

The procured liver is then placed in an appropriate organ-storage solution and triple-packaged in sterile packaging. This package should then be placed in another container that ensures a temperature of 2-8 °C and protects the recovered tissues during transport. Organ-perfusion machines are currently being evaluated for storage and transportation of liver tissue for organ transplantation and could potentially lead to a change in practice.

25.4. Processing and storage

Organs are transported to the designated isolation facility for processing. Depending on the size of the organ, the liver may be divided and perfused in parts. It has been reported that liver tissue preserves

12497 liver function better than isolated hepatocytes, so for repeated infusions of fresh hepatocytes (i.e. not
12498 cryopreserved) it may be better to isolate hepatocytes from different segments at different times to assure
12499 good perfusion and to minimise the time of isolated cells in suspension [15]. Vessels are cannulated to
12500 ensure perfusion of the liver tissue.
12501 The liver tissue is perfused in a 2- or 3-step procedure at 37-38 °C. First, buffer containing ethylene
12502 glycol tetra-acetic acid (EGTA) is pumped through the tissue to remove divalent ions, thereby disrupting
12503 cell–cell connections, then the EGTA is washed away by perfusion with buffer only. Finally, the tissue
12504 is perfused with collagenase/protease to digest extracellular matrix [8]. In some protocols, the second
12505 (wash-out) step may be omitted. Addition of the antioxidant N-acetylcysteine to the perfusion solution
12506 when isolating hepatocytes from fatty liver has demonstrated significant improvement in cell viability
12507 and metabolic function, and may be added for isolation of hepatocytes for clinical use. Isolated
12508 hepatocytes are purified by low-speed centrifugation. Cells that meet the release criteria after quality
12509 assessment (see §25.5) can be transplanted immediately after isolation, or cryopreserved and stored.
12510 Cells for transplantation are suspended in transplant medium (Plasmalyte or Eagle's minimum essential
12511 medium) containing 300 mM glucose heparin and human serum albumin (4 % final concentration) at a
12512 concentration of approximately 1-2 × 10^7 cells/mL [16].

12513 25.5. Quality controls/release criteria

12514 Hepatocytes exhibit a wide variety of functions that can be individually tested. Indeed, quality testing
12515 could be made appropriate to the recipient's disease, e.g. measurement of urea synthesis for recipients
12516 with urea cycle defects, or phase II conjugation activity for patients with Crigler–Najjar syndrome [17].
12517 However, when fresh hepatocytes are used, there is limited time for functional assessment before
12518 infusion. The most important quality-control tests are viability (should be > 50 %), as assessed by trypan
12519 blue exclusion, and number of cells. It should be remembered, however, that the trypan blue exclusion
12520 test detects only cell-membrane damage; it cannot detect apoptotic cells nor determine metabolic or
12521 physiological function. Functional tests should, however, be performed on aliquots of hepatocytes from
12522 the same batch used for transplantation, either in parallel or afterwards, for the evaluation of hepatocyte
12523 function for each batch/donor.
12524 Cryopreserved hepatocytes have the advantage that more extensive quality and genetic testing
12525 can be performed, which is not possible when using fresh hepatocytes due to time constraints. However,
12526 current cryopreservation protocols induce severe hepatocyte damage, which decreases both viability and
12527 function [9]. Testing should therefore be repeated after thawing of aliquots.
12528 The following tests could also be considered as quality-control tests:
12529 a. plating efficiency on coated plates (collagen, laminin, fibronectin or EHS matrigel), ability to
12530 attach to each other (spheroid formation);
12531 b. enzyme activities (cytochrome P450 activities, conjugation of bile acids, metabolism of
12532 molecular probes such as EROD, PROD, CDFDA);
12533 c. synthesis (albumin, A1AT, bile acids, lipoproteins);
12534 d. urea cycle activity, metabolism of ammonia into urea;
12535 e. markers of apoptosis.

12536 Since none of these endpoints have specifically been demonstrated to correlate with engraftment
12537 or *in vivo* proliferation of hepatocytes, no specific assays can be mandated at this time. If such assays
12538 are conducted, the data from any individual assay should not be considered sufficient cause to exclude
12539 the use of the cells for a transplant. These assays will provide additional information on hepatic function
12540 that can be used in conjunction with additional data, including trypan blue exclusion, to help in the
12541 decision whether to use or not use cells for a transplant or to evaluate outcome.
12542 Limited testing (viability tested on trypan blue and sterility on Gram staining) is used when fresh
12543 cells are transplanted; however, subsequent analysis allows for retrospective data on sterility and
12544 function of the cells.

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25.6. Packaging and distribution

Hepatocytes can either be transported under hypothermic conditions (2-8 °C) or cryopreserved. Hepatocytes transported under hypothermic conditions should be stored in an appropriate preservation solution. Transportation time under hypothermic conditions should be kept as short as possible, because hepatocytes decrease in viability and function over time [15].

25.7. Administration of hepatocytes

Although few cases of intraperitoneal and intra-splenic administration have been described, the most common route of infusion is into the portal vein. Intra-splenic infusion has been used in cases of liver cirrhosis, where intra-portal infusion is not possible or too risky. Intraperitoneal infusions are used when temporary liver support is needed for bridging. Intra-portal infusion is the main cell delivery route for clinical HT with the portal venous system accessed by percutaneous transhepatic puncture or inferior mesenteric vein catheterisation [18].

There are two routes of infusion in the portal vein, either by percutaneous transhepatic catheterisation of a portal vein branch, or through catheterisation of an ileal vein by a mini-surgical approach. The transhepatic approach is now technically well mastered by radiologists but entails a risk of intraperitoneal bleeding and of portal thrombosis. Although both risks are low in patients with normal liver function (most hereditary metabolic liver diseases), they may be much higher in patients with liver failure and coagulation disorders (acute liver failure and acute-on-chronic liver failure). A complete study of coagulation disorders before administration and potential corrections of the coagulation disorders by intravenous infusion of fresh frozen plasma and platelets might be necessary. Clinical (intensive care unit) and radiological (ultrasonography) surveillance are required after the administration in order to detect intrahepatic haematoma, peritoneal bleeding or portal vessels thrombosis.

The ileal vein approach requires a McBurney incision. Catheterisation of a small ileal vein may be difficult in patients with unstable haemodynamic status.

Another risk of portal infusion of hepatocytes is obstruction of sinusoids by clumps of cells, resulting in an increase of the portal pressure [19]. A continuous monitoring of the portal pressure during the infusion should performed. Infusion rate must be slow: 1-2 mL/min [16]. It is followed by immuno-suppressive treatment.

25.8. Traceability

Records covering the complete procedure – from donor selection to recipient transplantation – should be kept at the tissue establishment (see Chapters 2 and 14). If the donor also donated other organs, special care should be taken to ensure traceability from the organ donor to all other organ and tissue recipients, and vice versa.

25.9. Biovigilance

For all relatively new clinical applications of human cells, documentation of all adverse events and reactions is of particular importance because we can learn from them. For example, the above-mentioned lack of *in vitro* endpoints that correlate with engraftment or proliferation of hepatocytes *in vivo* will only be clarified after collecting sufficient data as well as monitoring adverse events during procurement and processing of hepatocytes (see also Chapter 16 for management of adverse reactions).

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Chapter 26. Adipose tissue

26.1.Introduction

Autologous fat transplantation in aesthetic and reconstructive plastic surgery has revolutionised surgical treatment for soft-tissue defect correction or volume augmentation in recent years. In 1893, Neuber reported the first autologous fat grafting [1]. With the invention of liposuction in 1977 and the proposed technique of reinjecting aspirated fat in the late 1980s [2], lipofilling procedure has become one of the most popular procedures performed by plastic and aesthetic surgeons [3]. Nowadays, the most common method of adipose tissue procurement and transplantation is Coleman's technique from 1994 [4].

Unlike with synthetic materials, there is no risk of rejection and the implementation costs are reasonable. Autologous fat transplantation can be used in both aesthetic and reconstructive plastic surgery for soft-tissue augmentation. In addition, it does not induce an immune response in the recipient and, as a filler material, is abundantly available.

Primarily, procurement, banking and transplantation of autologous adipose tissue should non-restrictively be supported in cases of reconstructive indications (e.g. Romberg's disease, depressed scars, eyelid depression, pitting acne, post-traumatic defects, subcutaneous adipose atrophy of senility, breast reconstruction, improvement of function and appearance of irradiated tissues, correction of asymmetry in Poland's syndrome, soft-tissue defect correction), though results after autologous fat transplantation are better in cases of aesthetic indications (e.g. wrinkles, volume augmentation), probably due to better recipient site condition [2].

Autologous fat transplantation represents a simple solution to restoring the profile of the breast during reconstruction procedure. In fact, in breast cancer surgery, lipofilling is usually used for the correction of defects and asymmetry following tumour excision [5]. Adipose tissue is preferred over other types for the correction of volume and contour defects because fat is autologous, abundant and easily procured [6].

One of the most common indications for this therapy is reconstruction after tissue removal in patients with breast cancer. Other indications for this procedure can also include lipodystrophy due to acquired immune deficiency syndrome (AIDS), so positive results in infectious disease testing can be accepted, as the tissue will be for autologous use. For more than 100 years, autologous fat transplantation has been used to correct subcutaneous lipoatrophy, resulting from hemifacial atrophy, acne, trauma, lipodystrophy and sclerodermia, cutaneous lupus erythematosus and defects resulting from accident, infection or surgery. Adipose tissue has been used in post-mastectomy pain syndrome; in fact, breast-conserving surgery has become a well-established alternative to mastectomy in the treatment of breast cancer, providing a less invasive treatment [7]. Fat transplantation is efficient also for breast augmentation in patients suffering from micromastia, postexplantation deformity, tuberous breast deformity and Poland syndrome [8]. Adipose tissue has been used also for the correction of cicatricial ectropion [9] and for superior sulcus deformity [10].

Most of the clinical data obtained from adipose tissue transplantation are from patients receiving lipofilling directly after procurement in a one-step surgical procedure.

So far, the main obstacle to achieving favourable outcomes is its unpredictable long-term results due to the high rate of resorption in the grafted site, which means overcorrection of the treated zone or additional grafting including repeated procurement, leading to increasing cost and surgical risks as well as discomfort for the patient. There are several approaches to improving fat graft survival, including changes to procurement and processing techniques.

The initial isolated adipose tissue is composed of adipocytes and stromal vascular fraction (SVF) cells, which include adipose stem cells, preadipocytes, fibroblasts, vascular endothelial cells, and a

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12671 variety of immune cells [11]. It has become apparent through extensive research in the past decade that
12672 SVF cells and adipose stem cells might improve fat graft survival, largely through their angiogenic
12673 properties [12, 13].
12674 In order to avoid multiple procurements, protocols are developed to store adipose tissue in tissue
12675 establishments, sufficient for several treatments. In 2001 Shoshani *et al*. reported the successful frozen
12676 storage of adipose tissue for repeated fat injection in a domestic freezer [14]. One year earlier another
12677 group was less enthusiastic and advised against storage [15].
12678 Autologous adipose tissue from liposuction is being used increasingly in plastic surgery for
12679 reconstructive procedures. Some of the implanted tissue is resorbed, so surgeons treating large defects
12680 frequently apply a staged approach; its absorption rate has been reported to be 30-70 % [16, 17]. This
12681 approach can be facilitated by storing all or part of the tissue collected from the initial liposuction and
12682 implanting it during subsequent interventions. 'Fat banking' eliminates the need for repeated liposuction
12683 and, thereby, reduces cost and the risk of morbidity. However, the overall quality of the cryopreserved
12684 adipose tissue is still less ideal than the fresh one.
12685 The following generic chapters (Part A) of this Guide all apply to adipose tissue banking and must
12686 be read in conjunction with this chapter:
12687 a. Introduction (Chapter 1);
12688 b. Quality management, validation and risk management (Chapter 2);
12689 c. Recruitment of potential donors, identification and consent (Chapter 3);
12690 d. Donor evaluation (Chapter 4);
12691 e. Donor testing (Chapter 5);
12692 f. Procurement (Chapter 6);
12693 g. Premises (Chapter 7);
12694 h. Processing (Chapter 8);
12695 i. Storage and release (Chapter 9);
12696 j. Principles of microbiological control (Chapter 10);
12697 k. Distribution and import/export (Chapter 11);
12698 l. Organisations responsible for human application (Chapter 12);
12699 m. Computerised systems (Chapter 13);
12700 n. Coding, labelling and packaging (Chapter 14);
12701 o. Traceability (Chapter 15);
12702 p. Biovigilance (Chapter 16).

12703 26.2. Donor evaluation

12704 The criteria for donor selection to be applied are the criteria for autologous donation. The patient must
12705 be provided with sufficient information on the process (including the planned storage period and tests
12706 performed) and must sign an informed consent form.
12707 Additionally, it should be ascertained that donors do not have any major systemic diseases or lipid
12708 disorders, and that they are not underweight. If the adipose tissue is to be stored and not only used in the
12709 same surgical procedure, infectious disease testing must be performed for all autologous adipose tissue
12710 patients, as described in Chapter 5. Patients known to have HIV or hepatitis B/C can be accepted for
12711 autologous use. In this case, the tissues and cells must be labelled accordingly (e.g. 'caution: biological
12712 hazard') and stored separately or under special conditions. (For further details, see §9.2.8 and §9.2.9).

12713 26.3. Procurement

12714 Usually, adipose aspirates are only used for immediate autologous fat grafting; therefore, adipose
12715 aspirates obtained from liposuction are usually discarded because currently there is not a widespread
12716 and well-established cryopreservation protocol to store the aspirates.

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Risk assessment on the conditions of procurement, processing facilities and storage should be conducted, and appropriate mitigating actions should be taken to prevent cross-contamination. Particular attention should be paid to procurement conditions, because they support the initial quality and low bioburden of the adipose tissue.

Before surgery, the various adipose areas of the body are examined to identify natural fat deposits. The most common donor site is abdominal fat because it is one of the largest fat deposits. The second most common sites are the greater trochanteric region and the inside of the thighs and knee [5, 6].

Various procurement and preparation techniques have been introduced to obtain better and more reliable survival of adipose tissue. The fat tissue is usually procured with a specific cannula with negative pressure from abdomen, thighs and hip with Coleman technique [4, 16], but several techniques for procurement are currently being employed. Adipose aspirates are collected in a specific container (for example, a Luer-lock syringe) and should be transferred immediately (at a transport temperature of 4 C) to the processing unit.

26.4. Processing

There are several published protocols for processing adipose tissue, but there is no evidence to prefer one technique above another.

Tissue processing includes washing (e.g. 0.9 % NaCl [2]), centrifugation (e.g. 300-3400 rpm for 3 min [2]) or decanting, eventually antibiotic decontamination, controlled-rate freezing (≈ 1 °C/min) with cryoprotective agents and then storage < -140 °C (in vapour phase of liquid nitrogen) to preserve maximum viability.

It should be taken into account that adipose tissue is very sensitive to external treatment (centrifuge, processing methods and temperature). In particular, Moscatello *et al.* described the requirements for cryoprotectants and controlled freezing/storage, and listed components which can affect the viability of transplanted adipose tissue [18]:

- procurement → procurement method, source location, donor age;
- processing → wash solutions, centrifugation, disaggregation;
- storing → media, cryoprotectants, storage temperature;
- recipient bed → infusion solutions, growth factors;
- implantation → method, location, flexibility.

There must be written protocols for all procedures related to liposuction and tissue transfer to tissue-processing facilities. Appropriate measures should be taken to minimise the risk of microbiological contamination, including possibly the addition of an 'antibiotic cocktail' to the lipoaspirate.

26.5. Quality control

To cryopreserve adipose tissue, quality control is an essential issue. Adipose tissues must be processed under sterile conditions and in an aseptic manner. All biological tests should be performed as described in Chapters 5 and 10, if the tissue is processed (regardless of the location where this is done).

Quality control must include microbiological testing of each batch. Histology, cell-viability and functionality controls are highly recommended. Microbiological analyses of procured tissue, rinsing solutions and tissue after possible decontamination must be carried out according to the *European Pharmacopeia* (*Ph. Eur.*). In cases of positive results after decontamination, the adipose tissue should be discarded.

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12759 ## 26.6. Preservation/storage

12760 Literature clearly recommends the use of cryoprotectants when long-term-storage of adipose tissue is
12761 desired [19-25].
12762 Cryopreservation is one way to indirectly overcome the problem of absorption of the autologous
12763 fat graft results in repeated procurement procedures (with increasing cost and risks for the patient), as
12764 the patient only needs to undergo one procurement procedure. Unfortunately, an optimal technique for
12765 long-term preservation of adipose tissues is not available, and outcomes following implantation are
12766 mixed. Further studies need to be done in order to develop a better cryopreservation method for long-
12767 term preservation. The selection of cryoprotective agent is one of the key issues for obtaining optimal
12768 viability of adipocytes.
12769 The most common cryoprotective agents used for adipose tissues are dimethyl sulphoxide
12770 (DMSO), trehalose [26, 27] and glycerol. A higher recovery of adipose tissue after cryopreservation can
12771 be achieved if cooling is performed slowly and thawing is done by fast warming [28, 29].
12772 Additionally, Hwang *et al.* [30] described the storage of adipose tissue at −20 °C.
12773 When autologous adipose tissue is procured and stored, positive test results will not necessarily
12774 lead to discarding the tissue. For such tissues, isolated storage possibilities should be considered in order
12775 to exclude risk of cross-contamination or mix-ups.
12776 A state-of-the-art research or validation study on the shelf life of preserved adipose tissue should
12777 be performed in order to determine the expiry date according to the used processing and preservation
12778 protocol.
12779 Processed adipose tissue should be kept below −85 °C, but preferably in the vapour phase of liquid
12780 nitrogen (<−140 °C). The thawing protocol must be gentle, when removing the cryoprotective agent as
12781 well the amount of free lipids and debris associated with loss of adipocytes.

12782 ## 26.7. Biovigilance

12783 Any adverse reaction or event occurring during procurement, processing, thawing or reinjection of tissue
12784 must be notified, as described in Chapter 16.
12785 Serious adverse reactions for adipose tissue transplantation include:
12786 • graft failure (e.g. volume loss, calcification);
12787 • malignancy possibly attributable to the transplanted tissue (mainly due to cancer stem cells in
12788 the autologous transplant);
12789 • fat embolism.

12790 Serious adverse events include:
12791 • wrong tissue supplied for the intended surgical procedure;
12792 • tissue supplied was damaged or transported at wrong temperature;
12793 • tissue supplied beyond its expiry date.

12794 No entries have so far been found in the Notify Library (www.notifylibrary.org) for the banking
12795 of adipose tissue.

12796 ## 26.8. Developing applications

12797 Adipose tissue may also be a source of SVF or stem cells (see Chapter 32) and can be cryopreserved
12798 before the cells are isolated [31, 32]. A search for clinical trials using SVF in www.clinicaltrials.gov
12799 revealed that 15 studies are currently actively recruiting for patients. Most studies are conducted to treat
12800 osteoarthritis and rheumatoid arthritis. However, the conditions potentially treated with an SVF
12801 approach include musculoskeletal, neurological, immunological, cardio-pulmonary and immunological

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disorders, and soft-tissue defects. Similarly, adipose-derived stem cells isolated from SVF and expanded *in vitro* are under investigation for a whole range of diseases [33, 34].

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Chapter 27. Medically assisted reproduction

27.1. Introduction

This chapter refers to the medical procedures used to achieve pregnancy and live birth involving the identification, procurement (collection), processing and/or storage as well as distribution of at least one of the following reproductive tissues and cells: oocytes, ovarian tissue, sperm, testicular tissue, embryos. These procedures may be carried out using freshly collected and/or cryopreserved gamete(s), zygotes or embryos originated from the couple being treated ('partner donation') and also from gamete donors ('non-partner' or 'third partner' donation). For the procurement, processing and/or storage of ovarian and testicular tissue, we refer to Chapter 28 on Fertility preservation. These contexts are, in general, addressed separately due to the different risks involved. Ovarian stimulation or any other clinical procedure that does not involve gamete procurement is not addressed in this chapter.

Medically Assisted Reproduction (MAR) is also referred to as Assisted Reproductive Technology (ART). However, MAR is a broader term, which includes ART, but also includes ovarian stimulation and intra-uterine insemination of sperm, whereas the term ART refers only to procedures where *in vitro* handling of gametes or embryos is included.

MAR is carried out in centres specialised in treating patients with fertility problems. These centres are usually a combination of a tissue establishment and an organisation responsible for human application, bringing together a clinical team and a laboratory team in a multidisciplinary unit.

Procurement, processing and/or storage and the distribution of reproductive cells may also be performed in separate gamete cryobanks not connected to a fertility clinic. The recommendations in this chapter concern all institutions where reproductive cells are handled.

MAR comprises various procedures, such as:

- Processing of sperm for the purpose of intra-uterine insemination. Sperm provided by the partner, or originating from a non-partner donor, is processed and transferred to the uterus directly prior to the estimated time of ovulation;
- *In vitro* fertilisation (IVF), either conventional, whereby collected and prepared sperm and oocytes are co-incubated (so-called routine or standard IVF), or intracytoplasmic sperm injection (ICSI), whereby a single spermatozoon is injected into a mature oocyte. IVF involves procurement (collection) and processing of gametes, fertilisation, culture and transfer of embryos into the uterus. Oocytes and/or sperm might be provided by a partner or by non-partner donor(s);
- Cryopreservation and storage of gametes, embryos and/or gonadal tissue;
- Pre-implantation genetic testing (PGT) that uses genetic identification methods to diagnose or screen oocytes or embryos *in vitro* to exclude known inherited disease or chromosomal rearrangements incompatible with the birth of a healthy child. These methods include pre-implantation genetic diagnosis (PGD, now named PGT-M for monogenic/single gene defects, and PGT-SR for chromosomal structural rearrangements) and pre-implantation genetic screening (PGS, now named PGT-A for aneuploidy testing).

Procedures such as cryopreservation of gametes or gonadal tissue can also be used in patients with certain diseases (e.g. cancer, some chronic diseases) for whom treatment may be potentially harmful to their fertility. In those cases, long storage of their cryopreserved reproductive tissues and cells may be proposed to children, adolescents and male or female adults. This approach, called 'fertility preservation', is addressed in Chapter 28 and is also an option for fertility preservation for non-medical reasons. MAR treatments can also be proposed to couples at risk of transmitting a serious transmissible disease – e.g. human immunodeficiency virus (HIV) or hepatitis B and C viruses (HBV and HCV) – to

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12919 the partner and/or the child. These practices are applied only after risk assessment of vertical and
12920 horizontal disease transmission and taking into account the patients' health condition. In some countries,
12921 MAR can be undertaken in single women or homosexual female couples. In a few countries in Europe,
12922 under stringent conditions, surrogate motherhood is allowed for women without a uterus or with a non-
12923 functional uterus, or for male homosexual couples. Through insemination or embryo transfer, the
12924 surrogate mother carries and gives birth to a child for the intended parents.
12925 MAR is performed in most countries in Europe. Each year, the European Society of Human
12926 Reproduction and Embryology (ESHRE) publishes a report of activity in European countries, based on
12927 voluntary declarations. The latest published ESHRE data (from 2013) include data from 1169 clinics in
12928 38 countries and report 686 271 treatment cycles, including 474 666 IVF/ICSI, 154 712 of frozen embryo
12929 replacement (FER), 40 244 of egg donation (ED), 247 of *in vitro* maturation (IVM), 9791 of PGT and
12930 6611 of frozen oocyte replacements. European data on intra-uterine insemination using
12931 husband/partner's semen (IUI-H) and donor semen (IUI-D) were reported from 1095 IUI labs in 22
12932 countries. A total of 175 467 IUI-H and 43 785 IUI-D cycles were included. In countries where MAR is
12933 well established, up to 6 % of children born per year were conceived using ART, and up to 9 % when
12934 including IUI [1].
12935 This chapter aims to provide guidelines that can help to conceive healthy singletons carried to
12936 term, which is the ultimate goal of MAR. The medical activities involved may in some countries be
12937 considered ethically sensitive. The procedures described here are intended to achieve efficient results in
12938 terms of delivery rates and also address the safety of patients, donors and children born. For partner
12939 donation, priority is given to using the reproductive cells from the partner even if the sample exhibits
12940 poor quality (e.g. ICSI for patients with decreased sperm quality) and/or at least one partner is infected
12941 by HIV or poses a high risk of transmission of a genetic disease.
12942 In addition, the following generic chapters (Part A) of this Guide all apply to MAR and must be
12943 read in conjunction with this chapter:
12944 a. Introduction (Chapter 1);
12945 b. Quality management, validation and risk management (Chapter 2);
12946 c. Recruitment of potential donors, identification and consent (Chapter 3, but only for non-partner
12947 donors);
12948 d. Donor evaluation (Chapter 4);
12949 e. Donor testing (Chapter 5);
12950 f. Procurement (Chapter 6);
12951 g. Premises (Chapter 7);
12952 h. Processing (Chapter 8);
12953 i. Storage and release (Chapter 9););
12954 j. Principles of microbiological control (Chapter 10);
12955 k. Distribution and import/export (Chapter 11);
12956 l. Organisations responsible for human application (Chapter 12);
12957 m. Computerised systems (Chapter 13);
12958 n. Coding, packaging and labelling (Chapter 14, but only for non-partner donors);
12959 o. Traceability (Chapter 15);
12960 p. Biovigilance (Chapter 16).

12961 This chapter defines additional specific requirements for MAR. Procedures may vary from
12962 country to country as determined by national legislation.

12963 27.2. Quality management, risk management and validation

12964 The implementation of a quality management system is mandatory and will contribute to compliance as
12965 well as to the success of a given MAR programme. This section should be read in conjunction with
12966 Chapter 2; however, certain MAR-specific matters concerning quality management are addressed below.

27.2.1. Risk-assessment analysis for laboratory activities

Risk management will help in assessing and prioritising the possible existing hazards in order to monitor and control them, so that the probability of an adverse event occurring will be kept to a minimum. The most commonly applied methods of risk assessment are: FMEA (Failure mode and effects analysis), FMECA (Failure mode, effects and criticality analysis) and Hazard analysis and critical control points (HACCP). (The process of risk assessment is described in §2.16.)

27.2.2. Validation

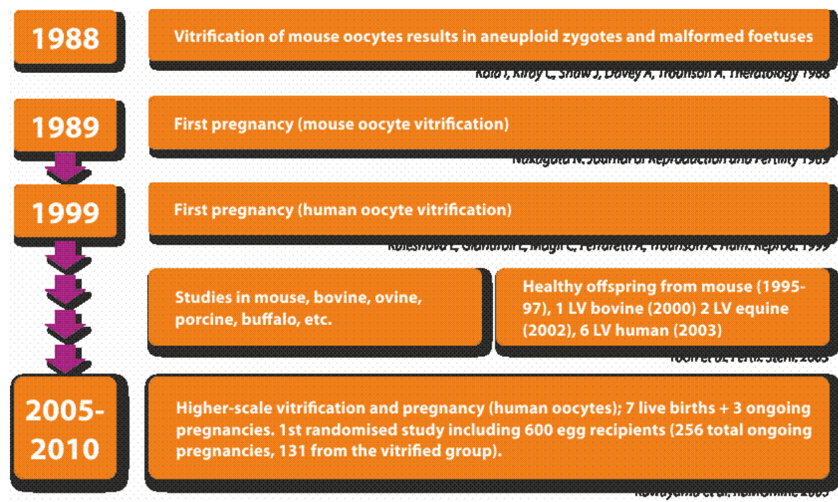
For MAR procedures, currently no test system with the necessary biological sensitivity exists, apart from an assessment of the actual clinical performance. Still, every piece of equipment and each new method should when possible be validated before it enters routine clinical use. Such validation should include installation, operational (technical) qualification and performance qualification, by comparison with laboratory key performance indicators (KPI) for similar types of equipment or methods already existing in the laboratory. The Special Interest Group in Embryology of ESHRE, in collaboration with Alpha Scientists in Reproductive Medicine, have established minimum performance (competence) levels and aspirational (benchmark) values for the IVF laboratory. Based on the information presented, each laboratory should select its own set of KPIs founded on laboratory organisation and processes [2].

Likewise, it is desirable to ensure that the necessary research and development has been conducted before bringing new techniques into clinical practice, preferably by means of well-designed randomised control trials with a follow-up of all children born from the procedure. The steps needed to validate a new technology before its introduction into clinical practice have been described (see Figure 27.1) [3, 4].

Figure 27.1. Validation of new technologies in assisted reproductive technologies

a. Theoretical steps to be followed when introducing a new technology into clinical use in assisted reproductive technologies

b. Example of a validation process in assisted reproductive technologies



Source: Adapted from Harper J, Magli MC, Lundin K *et al*. When and how should new technology be introduced into the IVF laboratory?" *Hum Reprod* 2012;**27**(2):303-13.

27.2.3. Materials, consumables and reagents

All consumables and media should be quality-controlled, fit for their purpose, of embryo-culture grade and, when available for the intended use and providing at least equal results as qualified alternatives, be CE-marked.

Specific culture media that fulfil the requirements of gametes and embryos are needed during all processing, fertilisation, culture, cryopreservation and other processing activities in MAR.

Patient or donor serum or follicular fluid should not be used as a protein supplement. Commercial suppliers of human serum albumin or media containing a serum-derived protein source should provide evidence of screening according to European and/or national regulations.

27.3. Recruitment of potential donors, identification and consent

27.3.1. Donor recruitment – non-partner donation

As with any tissues and cells, the donation of reproductive material should follow the principles of voluntary and unpaid donation, as described in Chapter 3 (see §3.2.1.2, which specifically relates to MAR). However, reimbursements for expenses related to the donation can be reimbursed, and loss of earnings may be compensated for.

National regulations will need to pay special attention to the existence of advertising and false or misleading promotion. In addition, the activities of tissue establishments related to donation should have a non-profit character, which means that only the actual costs of the additional services (those required to allow the donation to be performed) should be charged.

27.3.2. Donor consent – partner and non-partner donors

As mentioned above, MAR treatments can be undertaken with partner gametes or non-partner-donated gametes (i.e. 'partner donation' or 'non-partner' donation). Chapter 3 describes consent-giving procedures for donation by living donors, and this also applies to gamete and embryo donors in the case of non-partner donation. Also for partner-donated gametes, fully informed written consent is mandatory, and this section describes additional aspects of these specific consent forms that should be addressed. In MAR, consent forms may be separated for the female and male patients, although for certain treatments – and especially in partner donation – they could be combined in one document. It is important to

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emphasise that both partners need to sign these combined documents on partner donation. Examples of separate consent forms for treatment and storage are given in Appendices 11 and 12 for the female patient and in Appendix 13 for the male patient.

The couple (or individual) to be submitted to MAR treatment should receive written and oral information (during medical consultation with the physician or paramedical personnel, through information sessions, leaflets, website etc.) concerning the following:

a. national legislation about MAR and its implications for those who have access to assisted reproduction;
b. in cases of non-partner donation and embryo donation, the implications of current national legislation for the possible anonymity of the donor and for the possible right of the offspring to know their origins;
c. possibility of withdrawal of consent to treatment;
d. chances of success based on their medical history, the degree of invasiveness and the possible risks of the treatment (including multiple pregnancies); and, in treatments involving hormone administration, special reference must be made to ovarian hyperstimulation syndrome (OHSS) and the risks linked to oocyte retrieval (e.g. bleeding, infection or perforation of bladder or bowel);
e. testing for genetic and infectious diseases, and evaluation carried out in gamete donors in non-partner donation;
f. full description of the treatment at each stage of its implementation;
g. possibility of cryopreservation and storage of gametes and supernumerary embryos, and the options for future use according to national legislation;
h. total cost of the procedure, and existing reimbursement policies, if applicable;
i. possibility of the physician not proceeding with the entire treatment (or some of its parts) for medical or deontological reasons;
j. possible ethical issues regarding MAR;
k. possible psychological effects resulting from treatment using MAR;
l. possible risks to the offspring resulting from MAR treatment, particularly in multiple pregnancies, and the limited follow-up data on the long-term health of those born from the treatment;

In addition, psychological counselling should be offered to the couple/patient.

MAR treatment normally comprises a series of individual treatments, so consent forms should be signed for each treatment or else be valid for consecutive treatments until the treatment is successful, until a predefined date or until relevant circumstances change.

If the treatment is undertaken with cryopreserved gametes or embryos, consent for thawing should be given for each treatment. This policy would prevent a treatment from being initiated by either of the partners without the knowledge of the other.

In the consent form, the couple should state whether embryos or gametes may be cryopreserved or not. Depending on the possible options, the couple should receive information on the different success rates and on the implications of national legislation regarding the fate of cryopreserved gametes or embryos. The destiny of cryopreserved gametes or embryos could be: keep for own reproduction, donate to another couple, donate for scientific research or destroy. The consent form could also specify the duration of storage.

There should be specific consent whenever additional methods beyond IVF and cryopreservation are used. A very specific case in MAR is the possibility, in some countries, of consent for the use of the remaining gametes or embryos after one of the partners has died ('posthumous donation'). This needs to be clearly specified in the consent form.

A woman who enters IVF treatment could decide not to use all of her oocytes for her own treatment, but to donate some of them to other couples/individuals for reproductive purposes. This procedure is called 'oocyte sharing' and implies that this woman should be considered both a patient

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and a non-partner donor. Screening should therefore be conducted as described in Chapters 4 and 5, and specifically for ART in 27.5.2 and 27.6.2 below.

27.4. Donor evaluation

27.4.1. Evaluation of partner donors

27.4.1.1. Interview

Couples who experience problems in conceiving should be evaluated together because infertility constitutes a mutual medical problem for the couple. Counselling before, during and after treatment is widely practised and is recommended because fertility problems, investigation and treatment can be causes of psychological stress.

27.4.1.2. Taking of medical history and physical examination

Full medical history – including surgical, sexual, contraceptive, genetic, family and pregnancy history, as well as travel history for the assessment of certain viral diseases – should be taken from both partners. Both partners should also undergo a physical examination.

27.4.1.3. Screening of the female

Screening of the female should include:

a. assessment of ovulation, with a complete menstrual history; ovulatory dysfunction can be due to hypothalamic, pituitary or ovarian dysfunction;
b. assessment of ovarian reserve, including biochemical tests and ultrasound imaging of the ovaries;
c. assessment of tubal patency;
d. assessment of uterine abnormalities, such as submucous fibroids, polyps, adhesions or other Müllerian malformations (septae, bicornuate uterus);
e. testing for immunity to rubella should be carried out before treatment; vaccination should be offered to seronegative women before they commence any MAR treatment.

27.4.1.4. Screening of the male

a. at least one diagnostic semen analysis should be carried out before starting treatment; procedures and reference values are described in the *WHO laboratory manual for the examination and processing of human semen* [5];
b. men with azoospermia or severe oligozoospermia should be screened for genetic abnormalities (e.g. Klinefelter syndrome or Y-chromosome deletions) and, if a chromosomal abnormality is detected, appropriate genetic counselling should be offered; in the presence of obstructive azoospermia, cystic fibrosis or renal-tract abnormalities should be screened for; besides genetic testing, there should be hormonal testing and a scrotal ultrasound performed in order to establish a diagnosis of testicular failure.

27.4.1.5. Inclusion/exclusion criteria for treatment

A full medical evaluation will help to determine if a couple is suitable for MAR treatment. The risk–benefit analysis should be estimated on an individual basis.

The number of repeat cycles should be based on the individually estimated probability of a live birth.

27.4.2. Evaluation of non-partner donors

The purpose of evaluation in non-partner donors is to ensure that donors whose gametes may cause a health risk in the recipient or to the offspring (e.g. infectious disease, genetic disease) can be excluded. In addition, ensuring that the donation process does not cause harm to the health of the donor is equally important.

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To donate his/her sperm/oocytes, the potential donor must be submitted to:

a. consultation and counselling with a healthcare professional;
b. completion of a health/medical history questionnaire;
c. psychological assessment;
d. medical examination: gynaecological examination and ultrasound for female donors, and genital examination in males;
e. laboratory testing (including screening for infectious diseases);
f. ABO (AB0) blood group and rhesus typing;
g. genetic testing as indicated by family history and prevalence of carrier status in specific populations; karyotype testing is strongly recommended, and other extensive genetic screening for common recessive genetic mutations – like carrier testing for cystic fibrosis and spinal muscular atrophy (SMA) – is now available and should be considered in order to reduce the risk of transmitting genetic disease to the child;
h. semen analyses for sperm donors; freeze–thaw test may also be recommended, to assess the quality of the sperm after freezing and thawing;
i. assessment of ovulation and ovarian reserve (including endocrine work-up) in oocyte donors;
j. informed consent before any procedure.

27.4.2.1. Exclusion criteria for oocyte donors

a. age < 18 years or > 36 years;
b. positive results in tests for dominant genetic disease and/or infectious disease;
c. any risk factor to her own health;
d. unsuitability for donation based on interview.

27.4.2.2. Exclusion criteria for sperm donors

a. age < 18 years and > 45 years;
b. positive results in tests for dominant genetic disease and/or infectious disease;
c. poor sperm quality;
d. unsuitability for donation based on interview.

In cases in which embryos are donated, the partners of donors from whom the gametes originated must both be considered non-partner donors and must comply with the general criteria for non-partner donation in this section and in Chapters 4 and 5.

27.4.2.3. Psychological examination (non-partner donation)

In MAR, psychological evaluation of non-partner donors is highly recommended and should focus on a psychological anamnesis (including but not limited to: looking at motivation, looking at pattern of personal stability, discussing the psychological ramifications of being a gamete donor, giving psychological guidance in the preparations for becoming a gamete donor), often in combination with a personality and/or psychological diagnostic test.

27.4.2.4. Welfare of non-partner gamete donors

To secure the welfare of gamete donors is very important. Although the minimum age limit is 18 years, it could be good clinical practice not to include very young gamete donors, but to recruit an older donor group who have proved their fertility. It is important to counsel male and female non-partner gamete donors that the donors' DNA will be transmitted to any future children. Therefore, donating gametes may have a potential impact on the donor and his/her partner and family, including their own future children and their offspring. Although the donor can be unknown to the recipient, in some countries it is possible that the identity of the donor might be disclosed to the child when it is older. It is therefore also important, where applicable, to address the possibility of contact between future donor children and their gamete donors and to make sure that existing regulations about future contact are clearly described in the consent for donation.

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Regarding female donors, the risk of OHSS should be minimised, as it should be in all women submitted to MAR treatment. The number of times an oocyte donor may donate may be determined by several factors, such as the number of children and/or families achieved with this donor's gametes, the medical and psychological risks to the donor and the relevant legislation in the country of donation. Oocyte donors should preferably be accepted after having achieved a successful pregnancy of their own. Also for male donors, the number of donations should be determined by the number of children and/or families achieved with this donor's gametes, the psychological risks to the donor and the relevant legislation in the country of donation. Implementation of national registries for gamete/embryo donors as well as for recipients should be encouraged.

27.5. Donor testing

The purpose of testing gamete donors is to prevent transmission of severe infectious and genetic diseases from the donor to the recipient and their offspring, and to protect the staff while handling the patients and their gametes.

Testing of gamete donors is discussed here separately for each type of donation:

a. partner donation

b. non-partner donation.

Less strict biological testing is justifiable in the donation of reproductive cells between partners who have an intimate physical relationship (i.e. for partner donation).

27.5.1. Testing in partner donation

The following tests must be carried out:

a. anti-HIV-1 and anti-HIV-2;

b. HBsAg (HBV surface antigen) and anti-HBc (HBV core antigen);

c. anti-HCV.

Beyond these tests, tissue establishments should, based on analyses of risk or depending on stricter national legislation or recommendations, also carry out additional tests:

a. syphilis (a treponemal-specific test or a non-specific treponemal test can be used);

b. testing for human T-lymphotropic virus (HTLV)-1 antibody for donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or where the donor's parents originate from those areas;

c. additional testing may be required in certain circumstances, depending on the donor's history of travel/exposure as well as the characteristics of the tissue or cells donated, e.g. RhD (D antigen), diagnostic tests for malaria, Zika virus, *Cytomegalovirus*, *Chlamydia* and *Trypanosoma cruzi* (infectious agent for Chagas disease).

Blood samples for serology testing must be obtained before the first donation. In European Union (EU) member states, this must be done ≤ 3 months before the first donation. For further partner donations, additional blood samples must be obtained according to national legislation, but ≤ 24 months from the previous sampling.

Positive serology test results do not exclude donation between partners. Nonetheless, robust procedures should be in place to prevent the risk of contamination, to partner or to personnel, and of cross-contamination. If results for tests of HIV-1 and -2, HBV or HCV are positive, or if the donor is known to be a source of infection risk, a system of separate handling and storage must be put in place.

If the tissue establishment can demonstrate that the risk of cross-contamination and exposure to personnel has been addressed through validated processes, biological testing may not be required in the case of sperm processed for IUI and not intended for storage.

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27.5.2. Testing in non-partner donation

The following biological tests must be carried out for each donation:

a. anti-HIV-1 and anti-HIV-2;
b. HBsAg and anti-HBc;
c. anti-HCV;
d. syphilis (a treponemal-specific test or a non-specific treponemal test can be used);
e. in male donors: *Chlamydia trachomatis*. In the EU, this must be done from a urine sample by a nucleic acid test (NAT) but recent scientific data suggest ejaculate testing may be more sensitive.

In some cases, further tests may be required:

f. if required by stricter national legislation, e.g. in some countries, testing for HTLV-1/2 is mandatory;
g. testing for HTLV-1 antibodies must be done in donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or where the donor's parents originate from those areas;
h. additional testing may be required in certain circumstances, depending on the donor's history of travel/exposure and the characteristics of the tissue or cells donated, e.g. RhD – D antigen, diagnostic tests for malaria, antibodies to *Cytomegalovirus*, antibody to *Trypanosoma cruzi*, Zika virus infection. Latest epidemiological updates can be found at the European Centre for Disease Prevention and Control (http://ecdc.europa.eu/en/Pages/home.aspx).

All serum samples must be obtained at the time of donation. Sperm donations must be quarantined for ≥ 180 days after the last collection, after which repeat testing is required. If, at each donation, serology testing is combined with NAT for HIV, HBV and HCV, quarantine is not necessary unless further tests are required as mentioned in points f, g, h above. It is recommended that the same testing approach be used for oocyte donors, allowing for the safe use of cryopreserved or fresh oocytes if NAT is done at the time of donation. Oocyte donation could be considered as starting at the first day of stimulation, with the sample for testing to be taken at that time.

27.6. Procurement

27.6.1. Sperm

27.6.1.1. Collection by masturbation

Semen is usually obtained through manual stimulation or penile vibratory stimulation, or in rare cases through intercourse using a specially designed condom free of spermicidal substances. Patients should be given clear instructions regarding the collection of the sperm sample (hygiene, sexual abstinence, timing, etc.).

After thorough cleaning of the hands and genital area, semen is collected into a sterile collection container. The circumstances under which a semen sample is collected and delivered to the laboratory can influence the results of semen analyses. Since the time that spermatozoa are kept in the ejaculate can affect their survival, motility and fertilising ability, the start of diagnostic/therapeutic treatments must be standardised. If the sample can be collected in a special room adjacent to the laboratory, the risk of delays during transportation and cooling of the sample is minimised. This situation calls for appropriate design and equipping of the laboratory and semen-collection room. In general, patients are asked to collect a semen sample after 2-7 days of abstinence from ejaculation. Both too long and too short period of abstinence may influence the quality of the sample.

Semen samples should be collected into sterile, plastic containers (preferably tested for sperm toxicity). The use of spermicidal condoms, creams or lubricants must be avoided. The container should be clearly labelled, and correct identification should be confirmed by the patient. After collection, the

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sample should be delivered to the laboratory as soon as possible, avoiding extreme temperatures (< 20 °C and > 37 °C). Analysis should start within one hour of collection.

For traceability of samples to be used for treatment, records should be kept of the type of container used, the time of collection and the time interval between collection and analysis/preparation. The use of medication, fever during the previous months and completeness of the ejaculate collection should be documented.

27.6.1.2. Surgically retrieved sperm

In patients diagnosed with non-obstructive or obstructive azoospermia, sperm can be retrieved by surgical means from the testis or epididymis in an operating room. The method used is dependent upon the nature of the cause. This method may also be used for patients who cannot produce an ejaculate by masturbation.

27.6.1.2.1. Collection of sperm from the epididymis

Percutaneous epididymal sperm aspiration (PESA) is a method for sperm collection if the vasa deferentia are blocked. It involves the use of a sterile needle to aspirate sperm from the epididymis without a surgical incision. Both approaches typically yield sufficient quantities of sperm for ICSI, but not enough for a standard IUI or IVF.

27.6.1.2.2. Collection of sperm from the testis

An alternative to sperm collection from the epididymis is collection of sperm from the testis. This can be performed by testicular sperm extraction (TESE), and possibly by tissue removal (testis biopsy), and could be accompanied by a histopathology study for diagnosis. TESE can also be undertaken *via* a percutaneous approach – testicular sperm aspiration (TESA) – using a sterile fine needle or a biopsy needle. This is a less invasive procedure but usually results in less material than when TESE is undertaken.

27.6.1.3. Retrograde ejaculation

In cases of retrograde ejaculation, the sperm ends up in the urinary bladder after ejaculation. Although a procedure rarely used today, sperm can in these cases be collected from the urine after voiding, where the urine pH has been increased by intake of bicarbonates. Should this method yield very low-quality sperm, epididymal or testicular biopsies could be a better option for these patients.

27.6.1.4. Collection by electro-ejaculation

In some patients (e.g. in case of injury to the spinal cord, pelvic surgery, multiple sclerosis, diabetes mellitus with nerve involvement, unexplained anejaculation), ejaculation by masturbation is not possible. In these cases, ejaculation can be stimulated using a rectal probe with electrodes. This low-voltage stimulation is usually sufficient to produce a semen ejaculate. However, the quality of the ejaculate is often not as good as that obtained by masturbation. Also in this case, epididymal or testicular biopsies could represent a better option.

27.6.2. Oocytes

Before oocyte collection from the ovaries, also known as oocyte retrieval, the patient will be given hormonal treatment to stimulate the growth and maturation of the follicles in the ovaries (so-called controlled ovarian hyperstimulation or COH). During treatment, the patient is monitored closely to follow the response to the hormonal treatment and to assess the risk of OHSS.

Oocytes are collected through transvaginal ultrasound-guided ovarian puncture and aspiration of follicular fluid. The procedure can be carried out under local anaesthesia (paracervical block), sedation or general anaesthesia.

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13289
27.7. Processing

13290 Safety and quality issues covered in Chapter 8 also apply to the processing of human gametes and
13291 embryos. However, there are some specific issues that must be taken into consideration.
13292 The following section is based largely on *Revised guidelines for good practice in IVF laboratories*
13293 by ESHRE [6]. These guidelines were drawn up by the Special Interest Group (SIG) in Embryology,
13294 and constitute the minimum requirements for any laboratory performing MAR procedures.

13295
27.7.1. Premises for processing of gametes and embryos

13296 *27.7.1.1. Laboratory design*
13297 The laboratory handling gametes and embryos must have adequate space and should be as close
13298 as possible to the operating room in which clinical procedures are carried out. Laboratory construction
13299 must ensure aseptic and optimal handling of gametes and embryos during all phases of treatment. To
13300 ensure this, high-efficiency filtration of particulates and volatile organic compounds in the air supplied
13301 to the laboratory and rooms in which clinical procedures are carried out should be considered [7]. In
13302 addition, a number of protective measurements should be implemented to minimise the risk of
13303 contamination (see Table 27.1).
13304

13305 **Table 27.1. Criteria to be considered in determining the risk of culture contamination in assisted reproductive**
13306 **technologies processing facilities (EuroGTP guidance)**

Criterion	*Explanation*
Risk of contamination of tissues or cells during processing	Although very rare, accidental contamination from the environment during processing might occur. Working under oil in the laminar-flow hood minimises this risk.
Use of antimicrobials during processing	Use of antimicrobials during processing reduces the risk of contamination from the patient and/or the environment. Culture media for processing of oocytes, sperm and embryos usually contain antibiotics (e.g. penicillin, streptomycin, gentamycin).
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Destruction testing is not possible in partner donation or in non-partner oocyte donation. Therefore, in MAR, preliminary microbiological testing of donors before procurement and the methodology of processing are more important. Eventually additional testing of conditioned culture media can be of use to assess microbial contamination.
Risk of transfer of contaminants to patient	Working under oil in the laminar-flow hood minimises this risk. Cells in only a minimum amount of culture media are transferred into the uterus, so the risk of contamination is very low. Furthermore, other measures such as cleaning of the cervix on transfer will help to reduce the risk of infection.

13307
13308 According to the EU Tissues and Cells Directive, tissues and cell processing must be performed
13309 in a Good Manufacturing Practice (GMP) Grade A environment with a background of at least GMP
13310 Grade D. However, if it is detrimental or not feasible to carry out a specific procedure in a Grade A
13311 environment, or if a validated microbial inactivation process is applied, a less stringent environment
13312 may be acceptable. If so, an environment must be specified and it must be demonstrated and documented
13313 that the chosen environment achieves the quality and safety required.

13314 *27.7.1.2. Laboratory equipment*
13315 All equipment must be validated as fit for its purpose, and its performance must be verified by
13316 calibrated instruments; it should preferably be CE-marked.
13317 The laboratory must contain and identify all essential/critical equipment required for IVF, in
13318 numbers appropriate to the workload. Incubators in which gametes and embryos are cultured should be

13319 organised to facilitate their identification. The number of incubators is critical and should be based on
13320 the number of cycles and embryo-culture duration. Gametes and embryos should be conveniently
13321 distributed across incubators to minimise door openings and to maintain stable culture conditions.
13322 Devices for the maintenance of a constant temperature during manipulation of gametes and
13323 embryos that are out of incubators must be in place (i.e. warm stages, heating blocks). Regular checks
13324 of critical parameters such as temperature, pH related to CO_2 and O_2 levels must be carried out.
13325 A sufficient number of cryostorage units should be available and be continuously monitored and
13326 equipped with alarm systems, detecting any out-of-range temperature and/or levels of liquid nitrogen
13327 (LN_2).

13328 **27.7.2. Handling of gametes and embryos**

13329 As stated in Chapter 2, approved SOPs for all activities influencing the quality or safety of tissues
13330 and cells, including SOPs for handling of gametes and embryos, should be developed and maintained.
13331 Handling of biological material should be performed in laminar-flow hoods (Grade A
13332 environment) equipped with heating stages and pre-warmed heating blocks, using aseptic techniques at
13333 all times. Certain processes, such as ICSI and embryo biopsy, can be done outside the laminar hood
13334 since they need to be undertaken under an inverted microscope. Class-II hoods should be used for
13335 documented contaminated samples (e.g. HIV, HCV) since they provide protection to the operator.
13336 Measures must be taken to ensure that oocytes and embryos are always maintained at the
13337 appropriate temperature, pH and osmolality during culture and handling. Exposure to volatile or toxic
13338 substances, or harmful radiation, should be minimised.
13339 Pipetting devices must be used for one type of procedure only and must never be used for more
13340 than one patient. If possible, unit-dose sterile disposable pipettes are preferred. Each sample must be
13341 handled individually and its processing should be completed before moving to the next sample in order
13342 to prevent cross-contamination or mix-up of samples (see 27.13 on Biovigilance).

13343 *27.7.2.1. Oocyte processing*

13344 Oocyte retrieval is a particularly sensitive procedure, and special attention should be given to
13345 temperature and pH, as well as efficient and quick handling. An identity check before oocyte retrieval
13346 is mandatory. The time between oocyte retrieval and culture of washed oocytes should be minimal.
13347 Prolonged oocyte exposure to follicular fluid is not recommended. Appropriate equipment must be in
13348 place to maintain oocytes close to 37 °C. Flushing medium, collection tubes and dishes for identifying
13349 oocytes should be pre-warmed. Follicular aspirates should be checked for the presence of oocytes using
13350 a stereomicroscope and heated stage, usually at 8-60 × magnification. Exposure of oocytes to high-
13351 energy light should be minimised. Timing of retrieval, number of collected oocytes and identity of the
13352 operator should be documented.

13353 *27.7.2.2. Sperm processing*

13354 A test sperm preparation before starting a treatment cycle may be advisable in order to propose
13355 the most suitable insemination technique; a frozen back-up sample should be requested if difficulty in
13356 sperm collection on the day of oocyte retrieval is anticipated. Before starting sperm processing, an
13357 identity check is always mandatory. In the case of ejaculated sperm, the sample preparation aims to:
13358 • eliminate seminal plasma, debris and contaminants;
13359 • concentrate progressively motile sperm;
13360 • select against morphologically abnormal sperm.

13361 On the day of oocyte retrieval, an appropriate sperm-preparation method should be chosen,
13362 according to the characteristics and origin of the individual samples. The swim-up technique and
13363 discontinuous density-gradient centrifugation are most frequently used and widely accepted.
13364 In case of azoospermia on the day of oocyte retrieval, a second semen sample should be requested
13365 before considering alternative sperm-retrieval procedures or oocyte cryopreservation.

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For surgically retrieved sperm, several techniques are available to maximise sperm recovery and to select viable sperm among immotile testicular sperm cells [8]. In case of epididymal recovery, the aspirate is generally processed by swim-up or discontinuous density-gradient centrifugation, depending on the sperm cell number available. For testicular sperm, mechanical procedures to harvest the sperm from the tissue may be combined with enzymatic treatment in order to increase the sperm recovery rates.

27.7.2.2.1. Specific treatments

Although less often used, phosphodiesterase inhibitors (pentoxifylline, theophylline) or the hypo-osmotic swelling test are sometimes used in absence of motile sperm.

Enzymatic digestion of testicular tissue by collagenase may be applied if no sperm are observed.

27.7.3. Insemination of oocytes

Oocytes can be inseminated by conventional IVF or by ICSI. The insemination/injection time should be decided on the basis of the number of hours elapsed from ovulation trigger and/or oocyte retrieval, also keeping in mind that fertilisation will need to be checked 16 to 18 hours later.

27.7.3.1. Conventional in vitro fertilisation (IVF)

The number of progressively motile sperm used for insemination must be sufficient to optimise the chance of normal fertilisation. Typically, a progressively motile sperm concentration in the fertilisation dish between 0.1 and 0.5×10^6/mL is used.

The final sperm suspension should be in a medium compatible with oocyte culture.

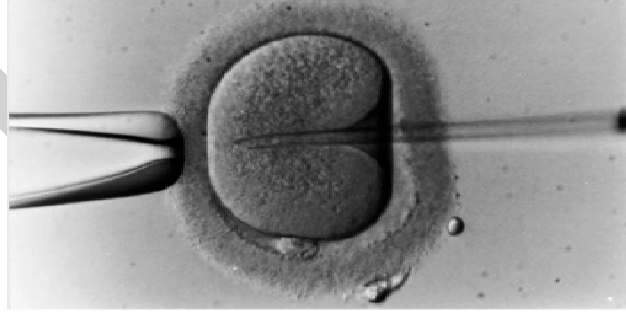
Co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a shorter period may be sufficient.

27.7.3.2. Intracytoplasmic sperm injection (ICSI) procedure

27.7.3.2.1. Preparation of oocytes for intracytoplasmic sperm injection

When removing cumulus cells from oocytes, hyaluronidase concentration and exposure should be kept to a minimum. In order to prevent oocyte damage, pipettes with appropriate lumen size should be used and vigorous pipetting avoided. After denudation, oocytes should be thoroughly washed to remove traces of hyaluronidase. The maturation stage of the oocytes should be recorded.

Figure 27.2. Intracytoplasmic sperm injection



Source: Image provided by María José De los Santos Molina (Spain).

27.7.3.2.2. The injection procedure

See Figure 27.2. It is recommended to keep records of the injection time (start and end of the procedure) and the performing operator. The duration of sperm identification and immobilisation followed by injection should be minimised. The number of oocytes transferred to the injection dish should relate to the operator's skills and the sperm quality. Appropriate temperature and pH should be maintained during injection. Viscous substances such as polyvinylpyrrolidone (PVP) can be used to

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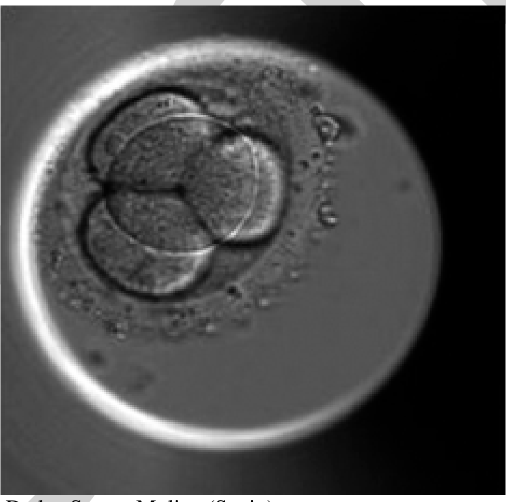
13403 facilitate sperm manipulation. In case of only immotile sperm cells, a non-invasive vitality test can be
13404 used to select viable sperm for injection. After injection, oocytes should be washed prior to culture.

13405 **Figure 27.3. Zygote with 2 pronuclei and 2 polar bodies**

13406
13407 *Source:* Image provided by María José De los Santos Molina (Spain).

13408 **Figure 27.4. Embryo at the 4-cell stage**



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13410 *Source:* Image provided by María José De los Santos Molina (Spain).

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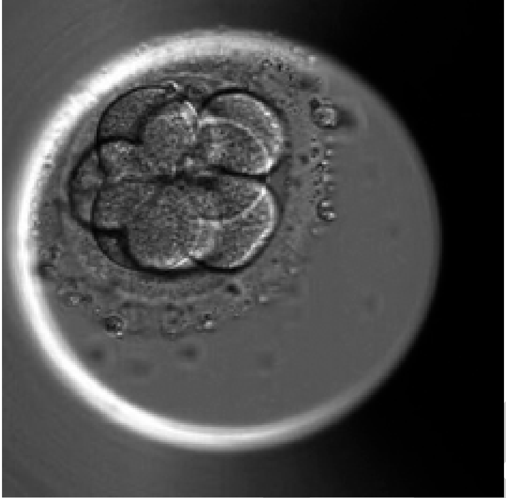
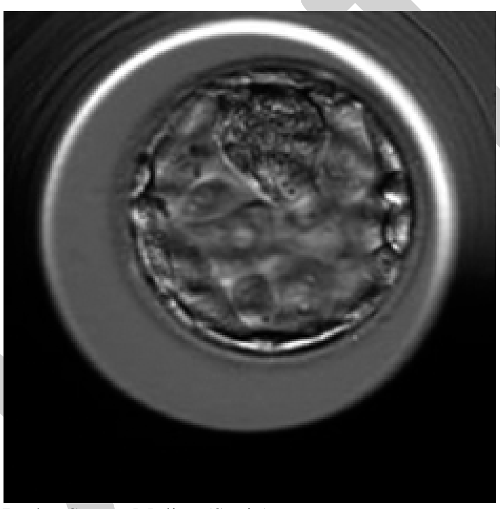
13411 **Figure 27.5. Embryo at the 8-cell stage**



13412
13413 *Source:* Image provided by María José De los Santos Molina (Spain).

13414 **Figure 27.6. Embryo at the blastocyst stage**

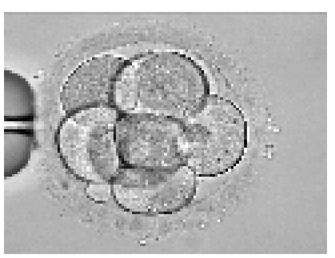
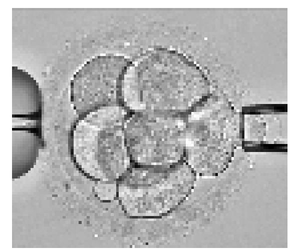
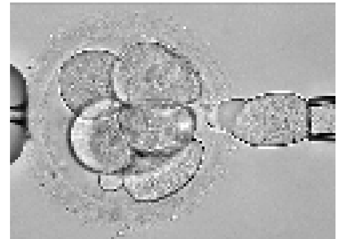
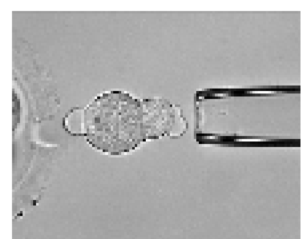


13415
13416 *Source:* Image provided by María José De los Santos Molina (Spain).

13417 **27.7.4. Assessment of fertilisation**

13418 All inseminated or injected oocytes should be examined for the presence of pronuclei (PN) and
13419 polar bodies at 16 to 18 hours post-insemination. A normally fertilised oocyte (zygote) contains 2 PN and
13420 2 polar bodies (Figure 27.3). For conventional IVF, cumulus cells must be removed and 2PN oocytes
13421 transferred into new dishes containing pre-equilibrated culture medium.
13422

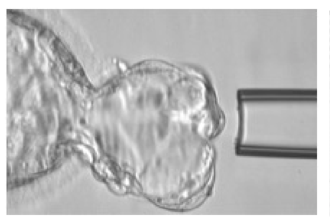
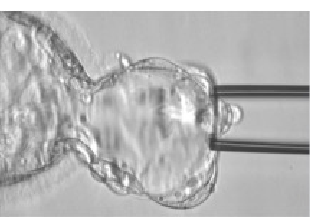
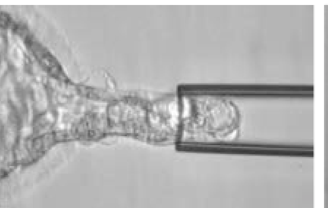
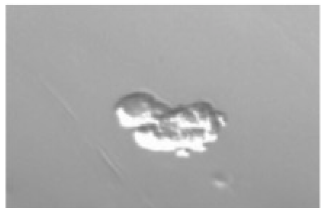
13423 **Figure 27.7. Embryo biopsy of an 8-cell stage embryo**

13424
13425 *Source:* Image provided by María José De los Santos Molina (Spain).

13426

13427 **Figure 27.8. Embryo biopsy of blastocyst**

13428 *Source:* Image provided by Cristina Magli (Italy).

13429

13430 Fertilisation assessment should be performed under high magnification (at least 200 ×), using an
13431 inverted microscope equipped with Hoffman or equivalent optics, in order to verify number and
13432 morphology of pronuclei.

13433 **27.7.5. Embryo culture and transfer**

13434 In order to optimise embryo development, fluctuations of culture conditions should be minimised.
13435 Precautions must be taken to maintain adequate conditions of pH, temperature and osmolarity, to protect
13436 embryo homeostasis during culture and handling.
13437 Embryo scoring should be performed at high magnification (at least 200 ×, preferably 400 ×) using
13438 an inverted microscope with Hoffman or equivalent optics. Evaluation of cleavage-stage embryos
13439 should include cell number, size and symmetry, percentage of fragmentation, granulation, vacuoles and
13440 nuclear status (e.g. multinucleation). Blastocyst scoring should include expansion grade, blastocoel
13441 cavity size and morphology of the inner cell mass and trophectoderm. Assessment should be performed
13442 at standardised times post-insemination. Embryo development can also be assessed using time-lapse
13443 imaging, allowing a dynamic evaluation of the timing of consecutive events during embryo culture.
13444 These systems also allow more stable culture conditions that may be of benefit. For an overview, see [9].
13445 Embryo-quality assessment records should include the operator(s), date and time of assessment,
13446 and embryo morphological characteristics, which should be noted with the developmental stage (see
13447 Figures 27.4, 27.5, 27.6).
13448 Embryo selection for transfer is primarily based on developmental stage and morphological
13449 aspects. Other selection parameters, such as time-lapse kinetics, may be considered.
13450 Single embryo transfer is recommended to avoid multiple pregnancies. The decision on the
13451 number of embryos to transfer should be based on embryo quality and stage of development, female
13452 age, ovarian response and rank of treatment. It is recommended never to transfer more than two embryos.
13453 Supernumerary embryos may be cryopreserved, donated to research or discarded, according to
13454 their quality, patient wishes and national legislation.
13455 If the laboratory is at some distance from the embryo transfer room, arrangements should be made
13456 to maintain temperature and pH while transporting embryos.
13457 A double identity check of the patient, the patient file and the culture dish(es) is mandatory
13458 immediately prior to the transfer.

13459 **27.7.6. Pre-implantation genetic testing**

13460 Oocytes and pre-implantation embryos can be biopsied and the obtained genetic material tested
13461 for certain monogenic disorders or chromosomal abnormalities. The biopsy procedure may be carried
13462 out by:
13463 a. removal of polar bodies;
13464 b. blastomere biopsy at day 3 (Figure 27.7);
13465 c. trophectoderm biopsy at the blastocyst stage (Figure 27.8).

Cell(s) destined for genetic analysis are removed in the IVF laboratory using glass microtools on a micro-manipulation set. The embryology laboratory has the responsibility of providing unique identification between biopsied polar bodies, blastomeres or trophectoderm cells and the corresponding oocyte, embryo or blastocyst, respectively. All cells and embryos for genetic investigation must be handled individually, avoiding DNA contamination from other cells, from the samples or from the operator. They must be identified and labelled carefully, and tracked during the entire procedure. During these steps, double identity checks are necessary. The biopsy sample should be subjected to diagnostic procedures in an accredited laboratory for medical genetics. Traceability for embryo identification must also be guaranteed during the analysis in the reference genetic laboratory.

The purpose of PGT-M (for monogenic/single gene defects) and PGT-SR (for chromosomal structural rearrangements) is to identify embryos generated *in vitro* that carry certain hereditary genetic diseases or chromosomal abnormalities and exclude those embryos from transfer. Due to a minimised risk of transferring affected embryos, these tests are an alternative to therapeutic abortion.

Genetic counselling must be available to all couples known to carry a (severe) hereditary disease. The recipient must be informed that due to mosaicism of the tested embryos and the limitations of the test, genetic testing on embryos does not substitute for prenatal analysis, such as amniocentesis.

PGT-A (pre-implantation aneuploidy screening) is used to analyse whether a cell biopsy from an embryo has the correct number of chromosomes, and such screening is used particularly for women of advanced reproductive age and for women who have had recurrent miscarriages or implantation failures. It is considered as a complement to standard morphological selection of embryos for transfer. Recent studies indicate that, for women of advanced reproductive age, embryo selection using PGT-A can decrease the number of embryo transfers necessary to obtain a pregnancy, thus reducing the time to pregnancy. However, the cumulative results are similar as when no PGT-A is used; see e.g. [10]. Bearing in mind the scarce data from prospective clinical trials and meta-analyses, PGT-A should be offered with caution, and full information on its present value should be provided to the patients.

Another possible future use for PGT could be to reduce the transgenerational risk of transmitting mitochondrial DNA disorder. Other utilisations such as the selection of histocompatible siblings can be also applied, case by case.

In some countries, PGT may not be allowed or allowed only in specific circumstances according to national legislation.

27.7.7. *In vitro* maturation

IVM refers to the maturation in culture of immature oocytes in specialised media after recovery from follicles that may or may not have been exposed to exogenous gonadotropins before retrieval [11]. During IVM, such oocytes progress from prophase I – i.e. from germinal vesicle (GV) stage – to reach metaphase II (MII). However, reaching the morphological criterion for MII (release of the first polar body) does not necessarily mean that the oocyte is competent for normal development.

Bearing in mind the lack of sufficient data from prospective clinical trials and meta-analyses, IVM should be considered an experimental procedure and not be used outside a system of ethical approval, and full information on its present value should be provided to patients.

27.7.8. Processing of samples from seropositive donors in partner donations

In couples with one or both partners being seropositive, MAR may still be applied for procreation, considering the risks of horizontal or vertical transmission of the infection, after appropriate counselling and with the informed consent of patients.

For couples with seropositive males, the process includes density-gradient separation of the semen sample and optional swim-up.

Processing of samples from seropositive partner donors should be handled according to specific SOPs to protect personnel and avoid cross-contamination.

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13513 Hepatitis B-seronegative individuals with seropositive partners should be offered vaccination
13514 before ART [12].
13515 Good quality and safety laboratory practices in assisted reproductive technologies for
13516 serodiscordant couples must be in place and should include personal protection of patients and staff,
13517 protocols for risk reduction of cross-contamination and proper decontamination of the work area [13].

13518 27.8. Cryopreservation

13519 27.8.1. Methods for cryopreservation of human gametes and embryos

13520 Sperm, embryos and, more recently, oocytes are being cryopreserved for future use in MAR
13521 treatments (supernumerary embryos or oocytes, fertility preservation, non-partner donor gametes for
13522 banking). At present, the two most used methods for cryopreserving gametes and embryos are slow
13523 freezing and vitrification.
13524 Slow freezing is a method using programmed step-wise decreases in the temperature of the
13525 solution in which the specimen is cryopreserved. This usually involves specific computerised equipment
13526 and programmes for cooling different types of tissues and cells in solutions with cryoprotectant
13527 substance(s).
13528 Vitrification is an ultra-rapid cooling method that relies on very fast temperature drops (4 000-
13529 6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the
13530 specimen is cryopreserved without formation of ice crystals. This is a fast method that does not require
13531 special cooling equipment (although special consumables are required), and is performed with the
13532 addition of specific cryoprotectants in higher concentrations (compared to slow freezing) for shorter
13533 exposure times.
13534 There are significant differences in the sensitivity of different types of male and female gametes
13535 and different-stage embryos concerning the cooling process and cryoprotectant agents used [14, 15, 16,
13536 17].
13537 Concerns about the safety and quality of cryopreserved human gametes and embryos are raised
13538 regarding cell damage (disassembly of meiotic spindles, membrane rupture), toxic effects of
13539 cryoprotectants (anti-freeze substances), osmotic damage and incomplete dehydration, all having an
13540 effect on the cell viability [18].

13541 *27.8.1.1. Cryopreservation of sperm*

13542 For sperm, slow freezing is still the method of choice, but rapid cooling is a possible alternative
13543 [13, 14]. Freezing/thawing of human sperm is a well-proven technology. Sperm samples are usually
13544 cryopreserved in glycerol-based cryoprotectant solutions in cryovials or straws, frozen in a programmed
13545 cell-freezing device or incubated in liquid nitrogen vapour and then plunged in the liquid phase.
13546 Seminal plasma, immotile and damaged sperm can be removed (by sperm processing) before
13547 freezing to select a population of sperm with a better chance of survival. It is recommended to process
13548 testicular biopsy samples before freezing.

13549 *27.8.1.2. Cryopreservation of oocytes*

13550 In recent years, successful cryopreservation of MII human oocytes has been reported worldwide,
13551 with rapidly increasing success rates due to optimisation of protocols. These data strongly suggest that
13552 vitrification may be the method of choice in oocyte cryopreservation, since improved rates of survival,
13553 implantation and pregnancy have been obtained using this method compared to slow freezing [15, 16,
13554 17].

13555 *27.8.1.3. Cryopreservation of embryos*

13556 Zygotes, early-cleavage embryos, morulae and blastocysts have been cryopreserved successfully
13557 and used later for 'frozen embryo transfer'. Slow freezing or vitrification can be used, with

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vitrification/warming in dimethyl sulphoxide-based cryoprotectants resulting in better survival rates; see e.g. [17]. Exposure time to the cryoprotectant before vitrification is crucial and must be strictly respected.

27.9. Storage

Regarding cryostorage premises, the main aspects to be considered are location, ventilation and construction materials (e.g. flooring must be sufficiently durable for liquid nitrogen spill).

From a practical point of view the storage room with the liquid nitrogen tanks should be located close to the laboratory, so that the cryopreserved gametes or embryos can be easily, rapidly and successfully transferred to the storage room and into the liquid nitrogen tanks.

A low-levels oxygen sensor and adequate alarm system in case of liquid nitrogen leaks should be put in place for safety reasons. For electricity-dependent equipment, alarms etc., the storage facility should be part of the clinic's general emergency plan whereby, in case of loss of electrical power, a generator or uninterrupted power supply (UPS) system must be in place.

The type of construction materials should be similar to the ones used in the procurements and processing facilities, with smooth surfaces and easy to clean. A special consideration in the choice of construction materials is that the floor should be resistant to large changes in temperature caused by liquid nitrogen spills.

27.9.1. Storage limits

There is no scientific evidence that gametes, embryos and gonadal tissue, if kept under appropriate storage conditions, deteriorate after a certain time of storage; hence, they can be stored for long periods of time. Use of frozen sperm through assisted reproductive techniques has led to the birth of healthy offspring more than 20 years after initial storage [19], and successful storage over a long period for oocytes and embryos has also been published [20, 21]. However, at defined time points, contact with patients should be made to determine the destiny of their cryopreserved material. In some EU countries, national laws determine a maximum legal storage period. Patients must declare in writing the destiny of their reproductive material when this maximum storage period has ended (see also §27.3.2 on donor consent).

A periodic inventory of the cryobank is recommended, including cross-referencing contents with storage records.

27.9.2. Storage temperature

Optimal storage temperature is based on the type of tissue, cryoprotectant and freezing method used. However, a temperature <-136 °C for gonadal tissue, embryos and gametes is appropriate, and >-130 °C is detrimental to the survival and quality of the material frozen. Even though storage in liquid nitrogen or liquid nitrogen-vapours is common practice, it is important to ensure that the minimum temperature is also maintained when handling the stored samples (<-140 °C).

27.9.3. Storage devices

Several devices can be used to store reproductive material. Sperm can be stored in straws or vials, whereas gonadal tissue is stored mostly in vials. Embryos and oocytes are stored in straws, whereby one straw can hold one or more embryos or oocytes. It is, however, advisable to store only one embryo per straw to encourage single embryo transfer and to keep traceability between the quality of the oocyte or the development of the embryo frozen.

In the case of using straws for storage (and especially for storage of oocytes), open or closed systems can be used. Using open storage systems means that, at some point in the processing of reproductive tissues and cells, there is direct contact of the cells with liquid nitrogen. In a closed system, there is no direct contact between cells and liquid nitrogen.

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13602 **27.9.4. Cross-contamination during storage**

13603 Introduction of contamination in the storage vessel is due to human manipulations during
13604 processing. Viral and microbial agents may survive during long periods of time in liquid nitrogen.
13605 However, no reports have shown cross-contamination between these environmentally induced
13606 pathogens and the preserved reproductive material. Also, storage of reproductive material originating
13607 from patients carrying infectious diseases in liquid nitrogen has not led to cross-contamination of other
13608 frozen reproductive material residing in the same vessel [22, 23]. Even though evidence is lacking, it
13609 should be considered good laboratory practice to store reproductive material of patients with positive
13610 serology and negative serology separately. Vapour-phase storage containers have been proposed as an
13611 alternative to liquid nitrogen containers. Periodic thawing and cleaning of storage vessels is
13612 recommended for extending the lifetime of the vessel as well as periodic decontamination of viral and
13613 microbial agents.

13614 **27.9.5. Storage safety**

13615 Storage in liquid nitrogen or vapour nitrogen vessels is definitely the most common infrastructure
13616 used to store gametes, embryos and gonadal tissue. Cryopreservation and thawing of material is a daily
13617 process in a fertility clinic. Therefore, it is of the utmost importance that personnel working in the
13618 cryogenic room have received appropriate training on how to handle liquid nitrogen, and that they are
13619 aware of the potential hazards. Personnel must be equipped with specific protective garments (gloves,
13620 boots and goggles) and use special forceps for manipulation of straws.

13621 ## 27.10. Distribution, import/export

13622 Transport of tissues and cells within the EU is usually referred to as distribution (see Chapter 10). During
13623 transport of gametes and embryos, measures need to be taken to ensure the quality, safety and traceability
13624 of reproductive tissues and cells. Before transport, some specific actions need to be taken using the
13625 appropriate documents:
13626 • a signed transport agreement between expediting and receiving institutions;
13627 • presence of valid documentation (patients and sample identification, import/export permission
13628 when applicable, in accordance with legislation, biological test etc.);
13629 • a protocol for adequate sample handling during transport, storage and thawing;
13630 • a protocol of acceptance, checking for possible damage to container, for samples and patient
13631 identification and for presence of valid documentation;
13632 • signed consent for sample transportation by patients and/or by institutions.

13633 It is also necessary to consider and strictly control the conditions during the actual transport
13634 because cryopreserved material is highly sensitive to any fluctuations in temperature. See also Chapter
13635 14 and section 27.9.
13636 For export to and import from countries outside the EU, different requirements need to be met;
13637 for details see Chapter 10.

13638 ## 27.11. Packaging and labelling in assisted reproductive technologies

13639 As addressed in Chapter 14, the coding, packaging and labelling of tissues and cells have an important
13640 role during banking procedures. Packaging applies only to cryopreserved gametes and embryos in
13641 storage and transport. Frozen gametes and embryos are packaged and stored in straws/cryovials as
13642 described in section 27.9.3.
13643 Labelling is intended to identify gametes and embryos unambiguously. Labelling and
13644 identification systems may vary between centres and countries. As mentioned in section 27.7.2,
13645 procedures must be in place that ensure correct identification of patients at all stages of handling, using
13646 at least two points of identification (e.g. treatment number, name, colour code and/or date of birth) and

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should include at least the names of partners (when relevant) and date of processing. For frozen samples, colour coding of cryovials and straws should also be used.

At cryopreservation, documentation on biological material should include labelling of devices, cryopreservation method, date and time of cryopreservation, operator, embryo quality and stage of development, number of oocytes or embryos per device, number of devices stored per patient, location of stored samples (tank, canister). Cryo-devices must be clearly and permanently labelled with reference to patient details, treatment number and/or a unique identification.

At thawing, documentation on biological material should include thawing method, date and time of thawing, identity of operator and post-thawing sample quality.

27.12. Traceability

Identification of patients and traceability of their reproductive cells are crucial aspects in MAR treatments. Each IVF laboratory must have an effective and accurate system to uniquely identify, trace and locate reproductive cells during each procedural step. A proper identification system should ensure that the main characteristics of patients (or donors) and their tissues and cells, together with relevant data regarding products and materials coming into contact with them, are available at all times.

Proper training in traceability procedures for all laboratory staff is highly recommended.

Before commencing any procedure, the laboratory must be provided with each patient's unique identification, which has to clearly and easily refer to the patient's documentation. Each treatment cycle must be assigned a unique identification.

Corresponding consent forms, clinical data and details of serological exams undertaken by patients/donors prior to admission to the treatment should be available to the laboratory staff.

Rules concerning the correct identification and processing of reproductive cells must be established in the laboratory by a system of codes and checks that considers all the following:

a. Direct verification of patient identity and correspondence with their assigned unique identification is required at every critical step. Patients should be asked to give their own identifying information (at least full name and date of birth) before procurement or assisted insemination/embryo transfer.
b. Labelling of dishes/tubes containing gametes and embryos must be permanent and on the container itself, not only on the removable lid.
c. All devices containing biological material must be clearly and permanently labelled with the unique patient and cycle identification.
d. Biological material from different patients must not be processed in the same working area at the same time.
e. Incubators and cryostorage systems should be organised to ensure easy access and identification of the biological materials therein.
f. During critical steps, traceability must be verified through correct identification of the reproductive cells and tissues. This can, for example, be executed by use of the four-eye-principle of witnessing (i.e. a double check of the identification by a second person) and/or by use of an electronic identification system.
g. Products and materials used with biological materials must be traceable. The date and time of each manipulation and the identity of all operators and witnesses must be documented throughout the treatment. These records should be kept for a specified period of time according to European and/or national legislation.
h. Gametes and embryos from non-partner donation require specific coding for those countries that are regulated according to European Commission directives, specifically Directive 2015/565 amending Directive 2006/86/EC (see also §14.2.3).
i. Transport of reproductive cells and tissue requires identification of distributing, importing and exporting institutions, as well as identification of the biological material and its conformity for clinical use. At both institutions, the accompanying documentation and sample identification on the storage device must be checked to ensure that they correspond with patient records.

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j. Tissue establishments that store and distribute non-partner gametes should label containers with an appropriate unique donation identification. In the EU, the coding requirements for non-partner donation apply (see §14.2.3.1-2).

27.13. Biovigilance in medically assisted reproduction procedures

Deviations from SOPs in tissue establishments or other adverse events that may influence the quality and safety of tissues and cells should result in SARE reporting to the Health Authority.

Examples of SAREs reported for MAR are given below. In addition, the Notify Library (www.-notifylibrary.org) includes many well-documented cases of adverse occurrences in MAR treatments. The database is publicly accessible and can be searched by substance, adverse occurrence and record number.

All patients involved should be informed as soon as possible and should be offered counselling and support.

27.13.1.Serious adverse reactions and events

27.13.1.1. Serious adverse events

Serious adverse events may be, for example, mix-ups or loss of gametes, embryos or tissues, and may occur at any stage of clinical or laboratory processes (collection, insemination, embryo transfer, cryopreservation). Reasons for mix-ups or loss of cells or tissues can be multiple processing steps, mislabelling, contamination, human factor involvement, misidentification, absence/failure of witnessing and/or poor-quality systems. The consequences may include reduced or no chance of pregnancy, (genetic) disease transmission, psychological impact and ethical/legal issues. Causal factors should always be investigated.

27.13.1.2. Examples of SAEs reported in MAR

27.13.1.2.1. Mix-ups/loss of traceability

a. mix-up of sperm samples during preparation/treatment;
b. sperm sample contaminated by another sample (e.g. with a used pipette);
c. oocytes fertilised with spermatozoa from the wrong person;
d. insemination of a woman with sperm cells from another couple;
e. wrong embryos thawed;
f. labelling error of tubes/dishes containing the oocytes/sperm/embryos.

27.13.1.2.2. Accidental loss of gametes and embryos

a. loss of gametes or embryos resulting in total loss of chance of pregnancy in one cycle (e.g. technical failure of incubator, cryomachine or cryotank, accident with culture dishes, accidental thawing);
b. embryos destined for culture or freezing were instead destroyed (error in transmission of information);
c. gametes or embryos lost due to microbiological contamination.

27.13.1.2.3. Adverse events after treatment with donated gametes

a. genetic condition discovered in a sperm/oocyte donor years after the gamete donation (for further information see section 27.13.2).

27.13.1.3. Serious adverse reactions

All serious reactions related to stimulation of the donor and procurement of the tissues and cells should be reported to the Health Authorities under the category of SARs in donors. Hospitalisation of oocyte donors due to ovarian hyperstimulation syndrome (OHSS) should be considered as an adverse reaction (non-serious adverse reaction if it is for observation only).

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Although the birth of a child with a genetic disease inherited from the donor is an affected offspring it should be reported under the category of SARs for recipients (see e.g. 27.13.1.3.2.d)

27.13.1.3.1. Examples of SARs reported in MAR for donors

a. severe OHSS leading to hospitalisation;
b. bleeding after oocyte retrieval;
c. ovarian torsion.

27.13.1.3.2. Examples of SARs reported in MAR for recipients

a. salpingitis after intra-uterine insemination;
b. bacterial infection of the recipient due to infected sperm;
c. ovarian torsion after ovarian stimulation;
d. mix-up of samples in the genetics or IVF laboratory (PGT-M treatment), causing the birth of a baby carrying a genetic disease.

27.13.2. Transmission of genetic diseases by medically assisted reproduction with non-partner donations

Donors may unknowingly carry genetic defects causing a (severe) disease. Thereby gamete banks, for example, when distributing sperm or oocytes from a non-partner donor to multiple recipients, could potentially be spreading a (severe) genetic disease. Tissue establishments should keep this in mind, especially when informing non-partner donors and recipients of non-partner donations. National registries to facilitate traceability of non-partner donors and offspring are strongly recommended.

Non-partner donors should be strongly advised to inform the procurement centre/tissue establishment if they are diagnosed with a genetic abnormality. It is recommended to contact the recipient in the case of a diagnosis that may seriously affect a child's health.

Recipients of non-partner donations should be advised to inform the clinic where they received fertility treatment and also any doctor treating a child with a genetic disease that the child was conceived through a non-partner donation, so that appropriate investigations about the origin of the genetic defect can be put in place. Measures should be put in place to prevent the use of gametes from the same donor until an appropriate investigation and risk assessment has taken place. Subsequent measures may include launching international rapid alerts if gametes from the same donor have been distributed or exported to other countries.

These examples emphasise that forward and backward traceability is of the utmost importance in MAR treatments.

27.13.3. Cross-border management of serious adverse reactions and events

Individuals travel abroad to access fertility treatment for various reasons (legal restrictions, long waiting times, treatment costs, lack of expertise, quality of treatment). If patients travel home after treatment, there is a risk that an SARE might occur that might not get reported to the professionals who carried out the treatment or to the Health Authorities. As a consequence, no investigation of potential causes is done and no preventive measures are taken. It is strongly recommended that medical teams involved in both countries communicate with each other to ensure adequate treatment and follow-up. Healthcare professionals should report any SARE to their national Health Authorities, even for cross-border treatments.

27.14. Additional considerations

Fair, clear and appropriate information must be provided to donors and recipients at all stages of MAR treatments. The chances of success (including the live-birth rate) should be discussed appropriately. Clinicians, embryologists, technicians, nursing staff and all involved professionals need to communicate at all times to ensure optimal teamwork for the benefit of patients.

OHSS risk, appropriate selection of laboratory methods, the risk of multiple pregnancy and its complications, and the need for follow-up of children must all be addressed. In this sense the use of a unique European database for donor–recipient allocation would be critical in achieving prompt, rapid and reliable SARE report management.

All establishments are strongly encouraged to document internal data and results, and to benchmark with international standards [1, 2]. It is also important to keep track of developments that may increase safety and quality.

In order to ensure global consistency and harmonisation when communicating regarding MAR, a consensus and evidence-driven set of terms and definitions has been generated [24].

27.15. Developing applications

MAR is a rapidly evolving field. Development and implementation of new technology may affect not only donors and recipients, but also future generations. It is therefore important that these procedures are proven to be safe and efficient.

A methodology for the introduction of new techniques and treatments into clinical practice has been proposed by Provoost *et al.* [4], involving three levels: from experimental, then innovative, to established. A scoring tool is used at each level to determine whether a threshold has been reached of sufficient efficacy, safety, procedure and effectiveness. (see Figure 27.9). For more information on the introduction of new methodology see Chapter 29.

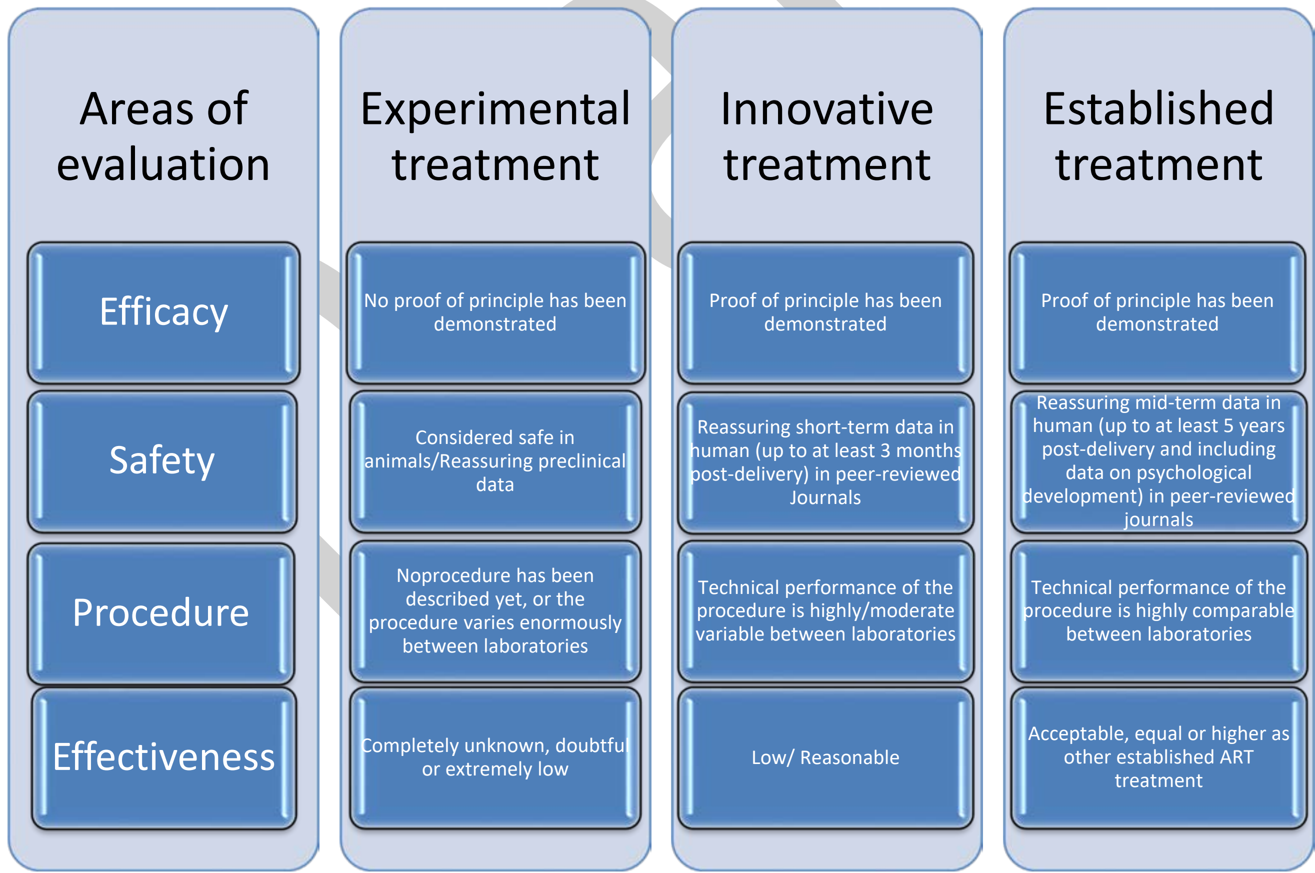

Figure 27.9. Experimental, innovative and established treatments

Source: adapted from Provoost V *et al*. Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014 Mar;**29**(3):413-17 [4].

13808 **27.15.1.Non-invasive pre-implantation embryo genetic testing**
13809 It is known that embryo morphology performs relatively poorly in determining embryo quality
13810 and predicting implantation. Many embryos that do not implant or end in miscarriage are indeed carriers
13811 of chromosomal abnormalities due to inherited meiotic and mitotic errors.
13812 The discovery of cell free DNA in human embryo-culture media supported the research on new
13813 non-invasive biomarkers of embryo viability that could eventually replace the current invasive pre-
13814 implantation embryo genetic testing (PGT) screening methods [25]. This new technology would be able
13815 to determine the euploidy status or even single gene mutations of human embryos by measuring cell
13816 free DNA in the spent culture media. Despite the fact that validation of the methods still needs to be
13817 undertaken, the techniques look very promising as recent studies have shown very good prediction
13818 power with high sensitivity and specificity values [26].

13819 **27.15.2.Whole genome screening**
13820 Recent research developments in the field of genomics have made possible the comprehensive
13821 testing of the human genome by combining the methods of next-generation sequencing with advanced
13822 bioinformatics. In this way, a complete picture of each individual genome, including single nucleotide-
13823 (SNV) and copy number-(CNV) variations, leads to expanded DNA screening. The application of this
13824 approach permits PGT and non-invasive prenatal testing, with expanded carrier screening, but also the
13825 disclosure of gamete donor anonymity. The comprehensive information derived from whole genome
13826 screening has benefits as well as limitations and risks, and its introduction into clinical practice requires
13827 prudence and genetic counselling [27].

13828 **27.15.3.Gene editing**
13829 Gene editing includes a group of technologies that allow modifying the genome by adding or
13830 removing genetic material at specific locations in the DNA sequence. CRISPR-Cas9 has been recently
13831 proposed as the most accurate, fast and economic among the available gene-editing techniques. In case
13832 of genes carrying a mutation, the enzyme cuts the mutation and replaces it with the correct DNA
13833 sequence, making it of great interest in the prevention and treatment of human diseases. This approach
13834 can be used for somatic gene editing for the treatment not only of a genetic disorder, but also of cancer
13835 and infectious diseases. Ethical concerns arise when gene editing is used to alter genes in gametes or in
13836 embryo, introducing changes that will be passed to future generations.
13837 In 2015, a study from China was published, reporting genome editing by CRISPR-Cas9 on non-
13838 viable human embryos [28]. The following year, the United Kingdom issued the world's first
13839 endorsement of a national regulatory authority for research on human embryos using genome editing.
13840 Additional studies have demonstrated how human germline gene modification is rapidly progressing
13841 from the experimental field to clinical research applications [29].
13842 The results obtained so far raise high expectations regarding possible therapeutic applications in
13843 humans, but much remains to be considered before clinical applications, including the reproducibility
13844 of the technique and possible long-term consequences.

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Related documents:

Appendix 11. Example consent form to treatment and storage (female)

Appendix 12. Example consent form fertility cryopreservation (female)

Appendix 13. Example consent form to treatment and storage (male)

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Chapter 28. Fertility preservation

28.1. Introduction

Despite some differences in technical approaches and expected results, today fertility preservation (FP) can be applied for both medical and non-medical (also known as social) reasons.

FP involves actions taken in order to avoid, delay, diminish or circumvent the exhaustion of the germ-cell pool of the individual. In most current circumstances, either in anticipation of cytotoxic therapy treating a severe disease or for a number of possible reasons for postponing parenthood, this involves cryopreservation of gametes, gonadal tissue or embryos.

FP techniques are usually proposed to males and females of reproductive age at risk of losing their reproductive potential due to either malignant or non-malignant diseases. Gamete cryopreservation is also an option for individuals for non-medical reasons, such as to postpone parenting, previous to vasectomy or other reasons.

This chapter describes the indications for male and female FP and the techniques actually available for the cryopreservation of reproductive cells and germinal tissue. The collaboration between paediatricians, oncologists and reproductive specialists is essential to ensure proper evaluation and counselling for each patient. Patient assessment and approach will depend on disease, age and treatment, and information about possible options and future use of cryopreserved gametes or germinal tissue should be discussed with patients, or parents (in the case of minors). It is important to realise that FP and restoration may include techniques that are in an experimental state, and their availability may be restricted according to national legislation.

This chapter must be read in conjunction with Chapter 27 on MAR (medically assisted reproduction) and the following chapters in Part A of this Guide:

a. Introduction (Chapter 1);
b. Quality management, validation and risk management (Chapter 2);
c. Recruitment of potential donors, identification and consent (Chapter 3, but only for non-partner donors);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);
h. Processing (Chapter 8);
i. Storage and release (Chapter 9););
j. Principles of microbiological control (Chapter 10);
k. Distribution and import/export (Chapter 11);
l. Organisations responsible for human application (Chapter 12);
m. Computerised systems (Chapter 13);
n. Coding, packaging and labelling (Chapter 14, but only for non-partner donors);
o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

28.1.1. Female fertility preservation

Female FP should be considered whenever fertility loss is predicted as a consequence of a cytotoxic treatment for a specific disease (e.g. in cancer patients) or due to the disease itself (malignant

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or non-malignant, e.g. severe endometriosis). This part includes indication for FP under medical conditions (oncological and non-oncological) as well as for non-medical reasons.

28.1.1.1. Medical reasons

All girls and women at reproductive age newly diagnosed with specific medical conditions (e.g. certain cancers or rheumatoid arthritis) whose treatment may cause premature ovarian insufficiency (POI) should be referred to a fertility expert to be counselled about the risk of infertility and informed about fertility preservation. FP should ideally be offered before treatment is started, but should not delay treatment. The risks of undergoing FP, including possible delay of the cancer treatment, should be weighed against the benefits of having reproductive cells and/or tissues stored for future use. Sometimes the patient's health may be too impaired by the disease to justify FP.

28.1.1.1.1. Oncological reasons for fertility preservation

Chemotherapy and radiation therapy may cause depletion of the pool of primordial follicles in the ovaries of girls or premenopausal women and thus render them infertile. Once the ovaries are exhausted of follicles, the patient will experience POI and infertility. In the case of pre-pubertal girls, loss of the entire stock of primordial follicles will mean that the girl will not enter puberty spontaneously and that she will not be able to become pregnant with her own oocytes later on in life. This is of course a very serious side-effect of an otherwise efficient cancer treatment, and is by many considered to reduce the quality of life significantly. As more and more girls and women at reproductive age survive a malignant disease today, these unwanted side-effects will affect an increasing number of adults in the population.

It is well known that chemotherapeutic drugs belonging to the group of alkylating agents cause the most damage to the ovaries. Alkylating agents, such as cyclophosphamide or busulfan, are used to treat various cancer forms, including breast cancer, lymphoma and sarcoma, and are also used in the preconditioning protocol before haematopoietic progenitor cells (HPC) transplantation. As alkylating agents cause damage to both dividing and resting cells, they are very toxic to the oocytes and granulosa cells of the primordial follicles, as this is the most immature and 'dormant' type of follicle [1].

Radiation therapy, whether given to the abdomen or the spine, will also affect the functionality of the ovaries. Radiation therapy is very toxic to the oocytes, and doses as low as 2 Gy will destroy half of the pool of follicles. Whenever possible, the ovaries are shielded or moved away from the field of radiation, but scatter dose is inevitable [2].

28.1.1.1.2. Non-oncological reasons for fertility preservation

Non-malignant diagnoses – such as kidney disease, autoimmune conditions or haematological diseases like aplastic anaemia or thalassaemia – can sometimes be life-threatening and require treatment with alkylating agents or even HPC. Women affected by any severe disease requiring these treatments may also need FP.

When surgery to the ovaries is planned, as in the case of severe endometriosis or benign ovarian cysts, or borderline cysts, healthy ovarian tissue containing primordial follicles will inevitably be excised in connection with the operative procedure. These procedures may pose some threat to the reproductive potential of the patient, and in these cases FP should also be offered [3].

Certain genetic conditions – such as Turner mosaicism, galactosaemia, Fragile X mutation carrier status or blepharophimosis, ptosis or epicanthus inversus syndrome – will cause premature exhaustion of the pool of primordial follicles in the ovaries, and girls and women at reproductive age suffering from any of these conditions can also be potential candidates for FP.

Several options exist to preserve fertility in post-pubertal girls and women. Oocytes, ovarian tissue and embryos can be cryopreserved depending on the characteristics of each individual case and considering the most efficient alternative for every patient. Examples of consent forms for female FP are given in Appendices 11 and 12.

Also cross-hormone treatment for transgender persons is potentially harmful to their fertility. Therefore, transgender female-to-male patients may undergo oocyte collection and storage before cross-hormone treatment and sex-reassignment surgery.

28.1.1.2. Non-medical reasons for fertility preservation

Non-medical FP can be also considered in young women who want to postpone maternity (age-related fertility preservation) [4].

In all cases the women should be aware of additional issues such as the expected survival rate of oocytes or the minimal amount of oocytes required to optimise the likelihood of successful live birth; in this sense, the creation of ad hoc prediction models is an interesting approach that may guide patients and clinicians [5].

Specifically, for age-related FP cases, the women should be adequately informed about the medical problems connected to any late pregnancies.

In the case of female-to-male transgender treatment, patients should be informed of the possible use of their gametes in the future. This approach may be different in different countries according to national legislation.

28.1.2. Male fertility preservation

28.1.2.1. Medical reasons

FP is indicated in all boys and men facing gonadotoxic treatment or surgical procedures affecting semen production and deposition. All patients at risk of fertility loss should be informed about FP options.

28.1.2.1.1. Oncological reasons for fertility preservation

Chemotherapeutic agents and radiation treatments can adversely affect the male gonadal epithelium. Thus therapies used to cure cancer (but also used for several non-malignant conditions) may render the patient temporarily or permanently infertile. The amount of damage depends on the regimen, the cumulative dosage of treatments used and the individual capacity of recovery. Dividing spermatogonia are highly sensitive to cytotoxic treatments and radiation. Low doses of these treatments deplete the pool of differentiating spermatogonia, while spermatogonial stem cells (SSC) may initially survive, and spermatocytes and spermatids can continue their maturation into sperm. Testicular involution occurs when no new precursors are provided from the stem-cell pool and the differentiating germ cells mature into spermatids and are released from the seminiferous epithelium [6, 7].

Significant damage is reported after treatment with alkylating agents, and different thresholds are given in the literature (e.g. for cyclophosphamide and cisplatin-based drugs). Both alkylating and platinum-containing agents cause direct DNA and RNA damage, and so can affect even non-dividing, reserve stem cells. The gonadal epithelium is highly susceptible to radiation-induced damage. Differentiating spermatogonia are sensitive to scattered doses of radiation as low as 0.1 Gy, leading to short-term cessation of spermatogenesis. Cumulative doses above 3 Gy affect SSC and cause long-term azoospermia, whereas doses in excess of 6 Gy deplete the SSC pool and can lead to permanent infertility. Fractionation of radiotherapy increases the germ-cell toxicity. Overall, post-treatment infertility problems are reported in up to 60 % of cancer patients [8].

28.1.2.1.2. Non-oncological reasons for fertility preservation

Likewise for male patients, certain non-malignant pathologies that require potentially gonadotoxic treatments could require FP. Transgender male-to-female patients may wish to store semen for FP. Transgender persons, planning to start cross-hormone treatment and undergo sex-reassignment surgery can benefit from FP. Analogous considerations previously explained for female–to-male trans patients also apply for male-to-female transgender patients.

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14047 28.1.2.1.3. Non -medical reasons for fertility preservation
14048 These indications include groups such as men in military services, who are at risk of potential
14049 harm to their fertility.

14050 28.2. Consent in fertility preservation

14051 After referral of the patient, informed consent for FP should be obtained by a clinician. However, since
14052 pre-pubertal children can also benefit from FP techniques, informed consent should in this situation be
14053 signed by the parents or legal guardians of the child. It is important that, in the case of FP for pre-pubertal
14054 children, care should be taken to explain the future use of banked gonadal tissue.
14055 Individual countries may have their own legislation regarding FP, and therefore consent forms
14056 can differ. An example of a generic consent form for cryopreservation of sperm is given in Appendices
14057 13 and 32. This form can be used as a template that can be adjusted according to national legislation or
14058 common practice of the MAR centre. Information with regard to the process, legal time of cryostorage
14059 and potential risks can be reported in the consent form or in a related information document.

14060 28.3. Patient evaluation

14061 Patient evaluation of post-pubertal women or men undergoing FP is similar to patient evaluation for
14062 patients undergoing MAR techniques (Chapter 27). The future use of the stored gonadal tissue or
14063 gametes is eventually their use in MAR techniques with the aim of obtaining embryos in a partner
14064 donation treatment.
14065 Patient evaluation for pre-pubertal boys and girls needs special care in cases where gonadal tissue
14066 is removed and banked. Close collaboration between paediatric, surgical, oncologic and fertility
14067 specialists is essential concerning FP in pre-pubertal children. When the patients are first seen in the
14068 oncology department, the influence of cancer treatment to the patient's future should be discussed and
14069 fertility and preservation options explained. The referral to a fertility clinic should be possible in very
14070 timely fashion because of the short time available for certain patients to undergo FP. In the fertility clinic,
14071 detailed information on the possibility and the process of FP will be offered on an individual patient
14072 basis. Additionally, the future use of the preserved reproductive material must be addressed. In order to
14073 accommodate the patients, certain aspects of the FP process can be considered: To minimise trauma to
14074 the patient, the surgical recovery of gonadal tissue should be combined with other interventions requiring
14075 anaesthesia, such as bone marrow sampling or implantation of venous ports. Close interdisciplinary co-
14076 operation between paediatric oncologists and gynaecologists, urologists, paediatric surgeons,
14077 psychologist or other medical specialist is required.
14078 In the case of pre-pubertal boys, measurement of testicular volume is helpful in predicting the
14079 chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen
14080 parameters – as soon as spermatogenesis has been induced – are comparable to those of adult patients,
14081 irrespective of the underlying disease [9-11]. In the case of pre-pubertal girls, the assessment of the
14082 ovarian reserve by anti-Müllerian hormone (AMH) may be investigated [12]. No further gynaecological
14083 investigations should be performed, since they can be perceived as intrusive and an emotional and
14084 psychological burden for these patients.

14085 28.4. Procurement

14086 28.4.1. Female

14087 *28.4.1.1. Ovarian tissue*
14088 Procurement of ovarian tissue can be performed at any time of the menstrual cycle and can be
14089 done at short notice.

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By the procurement of ovarian tissue, thousands of follicles can be preserved. The follicles lie within the cortical tissue of an ovary, with the vast majority of follicles in the outermost 1-2 mm of the ovary. An entire ovary, a semi-ovary or ovarian cortical biopsies are removed by an operative procedure under general anaesthesia and prepared for cryopreservation [13]. See Figures 28.1 and 28.2.

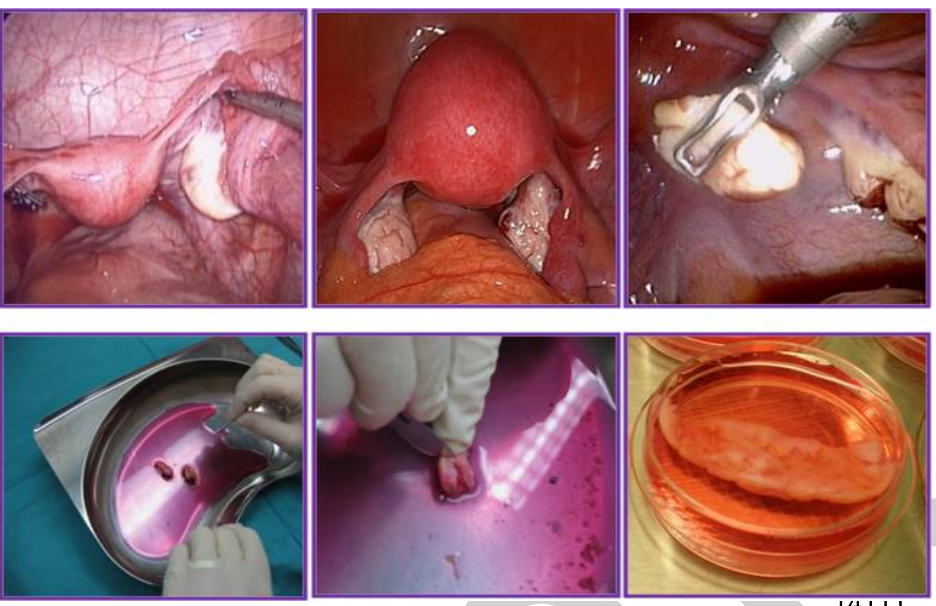

Figure 28.1. Ovarian tissue procurement by laparoscopy: stage 1

Note: Images in the upper panel show the surgery steps in obtaining the tissue. Lower panel shows the processing steps: the medulla of the ovary is removed.

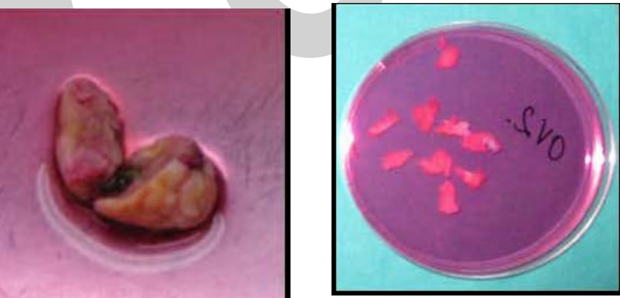

Figure 28.2. Ovarian tissue procurement by laparoscopy: stage 2

Note: After the medulla removal, the remaining ovarian cortex is then cut into small pieces of 10 × 5-10 mm.

Ovarian tissue procurement is offered to pre-pubertal girls, and to post-pubertal girls not ready to undergo ovarian stimulation, endovaginal ultrasound monitoring and oocyte retrieval in order to procure and bank oocytes. Adult women who do not have the time to undergo stimulation for procurement of oocytes, either because cancer treatment is imminent or because the cancer is hormone-dependent, are also candidates for ovarian tissue cryopreservation.

Although, technically, there is the possibility of storing ovarian tissue at the time of sex-reassignment surgery and thus during cross-hormone therapy, this approach is highly experimental [14]. For all patients, including transgender men, ovarian procurement should be performed before the start of gonadotoxic treatment.

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14136 *28.4.1.1. Oocytes*

14137 In order to collect oocytes, controlled ovarian stimulation is needed. This stimulation is similar to
14138 the stimulation for IVF (Chapter 27). The number of oocytes that can be collected depends on the age
14139 of the patient and her ovarian reserve. Special considerations are necessary to avoid high oestrogen
14140 production during ovarian stimulation in patients with oestrogen-dependent diseases. In cases of breast
14141 cancer, stimulation protocols have been developed to reduce the risk of an unwanted high level of
14142 oestradiol. Ideally, stimulation should start on the third day of the menstrual cycle, but can be started at
14143 any time in the menstrual cycle, including in the luteal phase, with apparently good results. Any pre-
14144 menopausal patient with a sufficient ovarian reserve can be considered for oocyte collection for FP. Post-
14145 pubertal girls may sometimes be able to undergo ovarian stimulation and tolerate endovaginal ultrasound
14146 monitoring and oocyte retrieval.

14147 Oocytes will be collected by aspiration via the transvaginal route following the same steps
14148 previously described in Chapter 27.

14149 **28.4.2. Male**

14150 *28.4.2.1. Testicular tissue*

14151 Testicular tissue is mostly procured in pre-pubertal boys when there is no possibility to produce a
14152 sperm sample. Collection of testicular tissue can be performed at any time. In general, unilateral
14153 procurement takes place, with a maximum of half of the testis.

14154 The procedure used for testicular biopsy in pre-pubertal boys is quite simple and similar to the
14155 technique described in adults. Basically, it should be performed at the cranial pole of the gonad, to avoid
14156 damage to the main testicular artery. After making a transverse or midline scrotal skin incision of 2-
14157 3 cm, the tunica vaginalis is opened and the lateral surface of the testis is exposed. The tunica albuginea
14158 is incised (0.5 cm in length) and the testes are squeezed to make the testicular tissue protruding. A biopsy
14159 of 2-3 mm^3 is then cut with scissors. The tunica albuginea and the skin are closed over. Besides being
14160 useful in fertility preservation, testicular biopsy in pre-pubertal boys is a minor procedure that can
14161 provide valuable information for predicting the risk of malignancy and fertility, as described in Faure *et*
14162 *al.* 2016 [15] (see also Figure 28.3).

14163 The amount of tissue procured for FP will have an effect on future testosterone production, and
14164 hormone replacement therapy could possibly be needed. However, it has been shown that the
14165 development of the testis in boys after biopsy of gonadal tissue for FP did not have an effect on the
14166 testicular growth [16].

14167 A balance between the amount of tissue retrieved and the amount conserved is important to
14168 achieve adequate levels of testosterone. Since the testis volume in very young pre-pubertal children can
14169 be limited, one third of the testis is generally procured in this patient population. Immunohistochemical
14170 staining is necessary to assess the presence of SSC in the procured and stored tissue [17].
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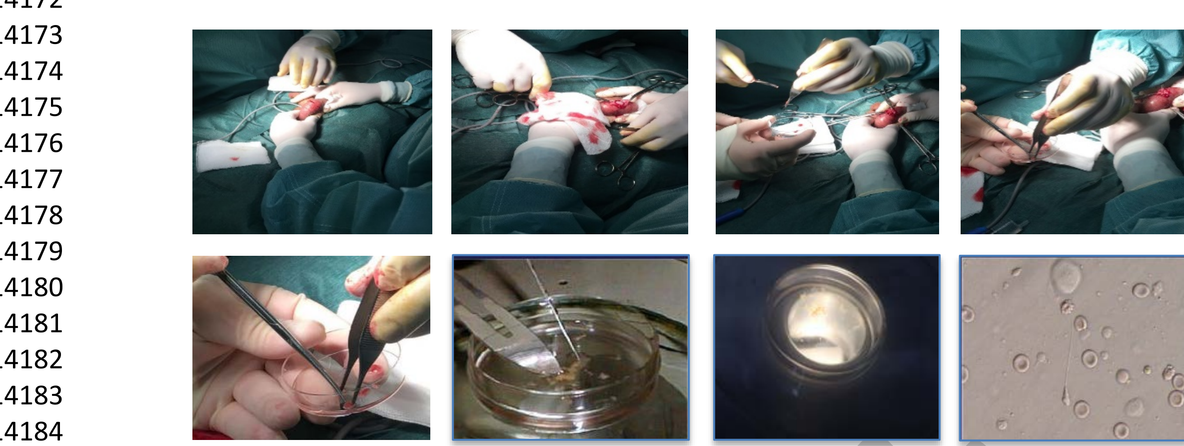

Figure 28.3. Conventional testicular biopsy and tissue processing for sperm recovery.

Note: Images in the upper panel show a surgical procedure for testicular tissue extraction. Lower panel shows processing steps: the small pieces of tissue are cut into smaller pieces. Isolated testicular spermatozoa and/or the tissue can be cryopreserved and thawed for future use.

28.4.2.2. Sperm

Sperm samples are mostly obtained through masturbation. Sperm samples can be collected in adult men, postpubertal boys and in peri-pubertal boys if the patient is ready to obtain a sample through masturbation [10, 18-20]. In cases of failure to produce a semen sample by masturbation, assisted ejaculation techniques such as penile vibratory stimulation or electro-ejaculation under general anaesthesia could be considered as a second-line treatment option.

Special care should be taken to clearly explain to young post-pubertal boys how to produce a sample by ejaculation, since not all patients are already sexually active.

28.5. Processing

MAR tissue establishments such as MAR centres and banks can process and store gonadal tissue, gametes and embryos for FP. The techniques for processing and storage are described in Chapter 27. Processing and storage of gonadal tissue require a tissue establishment with the facilities, licence and expertise to perform the procedure, and to process and store the tissue. These are described in more detail below.

Based on risk analysis, and offered testing for infectious disease (HIV, hepatitis) separate processing and storage of infectious material will be performed.

28.5.1. Female

28.5.1.1. Ovarian tissue

Ovarian tissue should be transported on ice in a transport medium (e.g. Leibovitz L-15), supplemented with serum albumin. Processing of the ovarian tissue starts with the ovarian biopsy or with bisecting the ovary, in the case of a whole ovariectomy. The medulla, the inner part of the ovarian tissue, is removed by careful scraping with a scalpel to prepare the cortical tissue to the required thickness of, on average, 1-2 mm. The cortex is subsequently cut into smaller fragments (5×5 mm). These fragments are then treated with a cryoprotectant (dimethyl, DMSO), to protect the cells from cryodamage, and generally subjected to controlled slow freezing in a programmable controlled-rate freezer [21]. Vitrification of the ovarian tissue is another optional methodology.

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14217 During ovarian tissue processing, the medulla should be further minced into small pieces in a petri
14218 dish with medium and examined under a stereomicroscope for the presence of cumulus oocyte
14219 complexes (COC). These COC can be collected and subjected to *in vitro* maturation in order to obtain
14220 metaphase II oocytes that can be collected and stored. This collection, with *in vitro* maturation and
14221 storing of oocytes obtained during the processing of ovarian tissue, is considered a highly innovative FP
14222 technique, since so far only one live birth has been described in Europe [22]. However, it opens the
14223 possibility of maximisation of FP in the case of ovarian tissue procurement and storage.
14224 Transport of the procured tissue from different centres to a centralised tissue establishment is a
14225 realistic and efficient system to be considered [21].

14226 *28.5.1.2 Oocytes*
14227 Oocyte cryopreservation is the preferred option for FP in post-pubertal patients who can be
14228 submitted to controlled ovarian stimulation. Vitrification is the technique of choice, due to the excellent
14229 results obtained in IVF patients in terms of survival, embryo development and implantation [23, 24].
14230 The methodology is described in Chapter 27.

14231 *28.5.1.3 Embryos*
14232 Although oocyte cryopreservation is generally practised today, embryo cryopreservation can also
14233 be considered for FP in the case of couples. However, cryopreserved embryos will not be available for
14234 future use if the couple separate.

14235 **28.5.2. Male**

14236 *28.5.2.1 Ejaculate*
14237 Sperm cryopreservation is performed for male FP in post-pubertal males. Semen characteristics
14238 may vary with both patient age and type of disease, with testicular cancer patients having the worst
14239 semen quality. For adolescents, in more than 80 % of cases semen can be cryopreserved. However, up
14240 to 20 % of adolescent or adult patients may either fail to produce a semen sample or may present with
14241 azoospermia. Measurement of testicular volume is helpful in predicting the chances of successful
14242 retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as
14243 spermatogenesis has been induced – are comparable to those of adult patients, irrespective of the
14244 underlying disease.
14245 The methodology for sperm cryopreservation is described in Chapter 27.

14246 *28.5.2.2 Testicular tissue/epididymus*
14247 For pre-pubertal boys and also for azoospermic patients, surgical sperm procured by testicular
14248 sperm-extraction procedures should be offered. Sperm can be retrieved by microsurgical aspiration of
14249 the epidydimal fluid or by testicular biopsy/testicular sperm aspiration (TESA) or testicular sperm
14250 extraction (TESE).
14251 Testicular tissue should be transported on ice in a transport medium (e.g. Hepes-buffered
14252 DMEM/F12), supplemented with serum albumin (in general, 10 % HSA).
14253 Processing of the testicular tissue consists of cutting the tissue into small fragments, submerging
14254 the pieces in medium supplemented with a cryoprotectant to protect the cells from cryodamage and then
14255 subjecting them to controlled slow freezing. However, no standardised protocol for cryopreservation of
14256 immature testicular tissue is available. Most groups are using DMSO-based cryoprotectants (0.7-1.4 M
14257 DMSO) with or without addition of sucrose. Slow-freezing protocols are mostly applied. Vitrification
14258 may also be effective when using higher doses of cryoprotectants. Vials/straws are thereafter submerged
14259 into liquid or vapour-phase nitrogen. Since the reproductive potential of cryopreserved immature
14260 testicular tissue has still to be demonstrated in humans, the technique remains experimental.
14261 The legislation and recommendations for FP in males differ between countries. There are no strict
14262 limitations on semen quality or sperm numbers for FP strategies and there are no international guidelines
14263 for the duration of storage of spermatozoa, whether ejaculated or procured directly from the testis.

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28.6. Storage

The permitted storage period of cryopreserved sperm, oocytes and embryos and reproductive tissues varies according to national legislation.

Long-term storage of ejaculated or testicular spermatozoa, ovarian cortex, oocytes or embryos does not negatively affect the quality of the frozen material, but constant storage conditions with a temperature of ≤−140 °C are mandatory [25-29].

28.7. Clinical application

28.7.1. Female fertility restoration

When a patient wants to use her preserved tissue or oocytes/embryos for MAR treatment, the physician who treated her with the gonadotoxic therapy should be consulted as to whether it is safe for the patient to attempt a pregnancy.

In case of transplantation of ovarian tissue, this can be done either orthotopically (at the remaining ovary or at the site of the removed ovary) or heterotopically to other sites such as the abdominal wall. It takes approximately 20 weeks from the time of transplantation for the tissue to become active again as demonstrated by the return of menses and oestradiol production. Hence, restoration of fertility is combined with the restoration of the patient's endocrine environment. Although the primary reason for the use of stored reproductive material may be a future desire for a child, the restoration of endocrine function could also potentially be a reason for transplanting ovarian tissue. The latter has to be considered with caution as a recent review by [30] has shown that endocrine restoration rate was 63.9 %.

Spontaneous pregnancies can occur after the follicles start maturing and the patient gets her cycle back, but sometimes IVF is needed. The duration of functionality varies from a few months to up to 10 years, with a mean of 3-4 years. If oocytes or embryos have been cryopreserved, an embryo replacement cycle must be planned. If she is menopausal, her endometrium will be prepared in a hormone replacement cycle.

It has to be emphasised that a significant percentage of women will experience a spontaneous return of ovarian function months after chemotherapy. For these women spontaneous pregnancies may occur and they may not need their frozen gametes or gonadal tissue [31].

Pre-menarchal girls who lose all their ovarian tissue due to chemo- or radiation therapy will not enter puberty spontaneously. These girls will need to be induced with exogenous hormones in order to undergo normal pubertal development. After puberty they will need to take hormonal replacement therapy for the health of their bones and for general well-being. Later on in life they can have their cryopreserved ovarian tissue transplanted in order to re-establish menstrual cycling and/or become pregnant.

28.7.2. Male fertility restoration

In most of the cases where chemotherapy and/or radiotherapy has been applied, spontaneous recovery of spermatogenesis is possible up to 10-15 years after the end of treatment; however, it cannot be accurately foreseen. Thus, regular semen analysis should be offered to patients after treatment. About 60 % of male cancer patients will face infertility problems after the end of the cancer therapy.

When cryopreserved samples are used, intracytoplasmic sperm injection (ICSI) is recommended to improve the chances of success. Before ICSI was implemented, the success rate of MAR procedures with cryopreserved semen samples (IUI or IVF) was low. When ICSI procedures are applied, the success rates using cryopreserved spermatozoa are comparable to standard IVF and ICSI procedures in infertile couples using fresh sperm.

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28.8. Quality control and tissue evaluation

Quality control after tissue transplantation includes approaches aimed at decreasing the risk of cancer recurrence while maximising the tissue viability after thawing or warming.

As the autotransplantation of cryopreserved tissue could be associated with a risk of cancer cell reseeding, due to malignant cell transmission in oncological patients, different approaches to detect cancer cells are under development [32-34]. Depending on the medical reason for tissue cryopreservation and the type of disease, the ovarian cortex and testicular tissue should be ideally sampled and sent for histological examination to detect any malignant cells.

Since cryopreservation methods can also affect tissue viability, examination of tissue survival and presence of primordial follicles can be performed. However, due to an uneven distribution of primordial follicles along the ovarian cortex, the inexistence of primordial follicles in the examined tissue should not prevent transplantation. The success of ovarian cortex transplantation can be evaluated by measuring the endocrine function and fertility restoration. Recent follow up studies have revealed an endocrine restoration rate of around 60-65 % and an ongoing pregnancy rate of 38 % after natural conception [30, 35].

28.9. Biovigilance

Any adverse event or reaction should be notified, based on the general rules described in Chapter 16.

28.9.1. Female

In certain kinds of cancers, transplantation of the cryopreserved tissue is not possible due to the risk of re-introducing the original disease. This is to be considered especially in the case of leukaemia, since it is known that leukaemic cells can reside in the stroma of the cortical tissue. Women suffering from disseminated cancer with a risk of ovarian metastases should be advised against transplanting the tissue [4, 36, 37]. Since such transplantations are scarce, compared with other disciplines, limited cases of adverse event and reactions have been reported. Up to date the surgical related complications remain low (-3 %). The report of a one major complication (intra-abdominal haemorrhage) has been recently published [35]

However, the reporting of serious adverse reactions and events affecting the offspring should follow the same rules used for medically assisted reproduction (Chapter 27).

28.9.2. Male

When cryopreserved sperm samples are used, the ICSI technique increases the number of MAR treatments that can be performed. No adverse effect on the health of the offspring has been reported from the combination of cryopreservation of semen and subsequent MAR.

A number of studies have been performed regarding sperm quality in the man after spontaneous recovery of spermatogenesis. Both cancer and its treatment are associated with sperm DNA damage, although treatment-induced DNA damage seems to be modest and transient. In a large cohort study of offspring from male cancer survivors, a modest but statistically significant increase in the risk of major congenital abnormalities was observed. This was independent of whether the sperm were cryopreserved pre-treatment and used for MAR, or if the children were conceived naturally [38, 39].).

Any report of serious adverse reactions and events should also follow the same criteria used for assisted reproductive technologies (Chapter 27).

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28.10. Future developments

28.10.1. Female experimental approaches

Female patients who seek FP but cannot undergo ovarian stimulation and oocyte/embryo preservation may consider using immature oocytes – either retrieved from antral follicles during the luteal phase or obtained during the ovarian cortex processing technique – to perform *in vitro* maturation to produce metaphase II oocytes to be used in ART [40]. Due to the lack of sufficient data from prospective clinical trials and meta-analyses, IVM should be considered an innovative procedure, and full information on its present value should be provided to patients.

In cases where no oocytes are existing, current investigations are carried out on the generation of oocytes derived *in vitro* from pluripotent stem cells as a promising though still incipient therapy.

Future techniques could also involve *in vitro* perfusion and hormonal stimulation of the patient's removed whole ovary(ies), where oocytes may be matured, aspirated and cryopreserved. This would enable fast oncologic treatment of the patient, as well as removing the risk of introducing malignant cells via transplantation.

28.10.2. Male experimental approaches

Development of the procedures used for the preservation of SSC and testicular tissues from boys and adolescents is far more advanced than research into the methods needed to realise the fertile potential of these cells. In principle, fertility restoration strategies in laboratory practice will include autotransplantation of a suspension of SSC by injection into the testis to restore spermatogenesis or autotransplantation of frozen-thawed testicular grafts back into the testis or an ectopic site. Should any risk of re-introduction of malignant cells exist via the transplant, then the only option is to grow and mature the SSC *in vitro*.

SSC transplantation was originally described in the mouse and is now an established research tool. SSC are infused through the efferent ducts into the rete testis, a technique which has been successfully applied in a number of species, including humans. The procedure is best performed under ultrasound guidance and presents a relatively non-invasive strategy for stem cell transfer. However, the colonisation efficiency after infusion of enzymatically digested testicular cells remains low. For future clinical applications, SSC need to be isolated, enriched and propagated *in vitro* before they can be autotransplanted in the numbers required to efficiently recolonise the testis and reinstate spermatogenesis. Nonetheless, the principle of the procedure has been shown and offspring have been generated from transplanted spermatogonia in a number of species, including primates [7]. While the demonstration of functional donor spermatogenesis following SSC transplantation in primates is an important milestone towards using SSC to restore human fertility, it remains vitally important to prove that the epigenetic programming and stability of SSC are not compromised following cryopreservation, culture and transplantation in humans.

Grafting of fragments of testicular tissue provides an alternative strategy to the use of cryobanked immature testis tissue. This approach maintains the SSC within their non-exposed natural niche, thus preserving the interactions between the germ cells and their supporting somatic cells. This procedure was successfully applied to retrieve sperm from ectopic and intra-testicular allografts, and insemination studies using ICSI have demonstrated that the spermatozoa were able to support full-term development of the progeny. This procedure is now tested in a number of species.

The major hurdle which must be overcome in patients with a haematological malignancy is the risk of re-introducing residual malignant cells via the testicular tissue. Sorting protocols using magnetic activated cell sorting (MACS), fluorescence activated cell sorting (FACS) or differential plating have been found to have variable efficiency when used to enrich human SSC. The risk of re-introduction of malignant cells via the graft may be circumvented by *in vitro* spermatogenesis. *In vitro*-derived spermatozoa that are free from residual disease can then be used to inseminate oocytes using ICSI.

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14394 Various strategies – including standard 2D cultures, 3-dimensional culture of testicular cells or organ
14395 culture – have been tested and showed some promise [41]. Although encouraging results have recently
14396 been obtained regarding the genetic and epigenetic stability of human SSC during long-term culture, the
14397 fertility of *in vitro*-derived sperm has still to be established before the clinical value of this type of
14398 experimental approach can be fully assessed.
14399 Similarly as in the case of oocytes, when no germ cells are available in the initial testis biopsy, an
14400 alternative option may be the *in vitro* derivation of sperm cells from the patient's somatic cells, such as
14401 skin fibroblasts, by induced pluripotency or transdifferentiation of these cells. This approach is,
14402 however, still in its infancy.

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14509 **Related documents:**
14510 **Appendix 11. Example consent form (female);**
14511 **Appendix 12. Example consent form (female);**
14512 **Appendix 13. Example consent form (men);**
14513 **Appendix 32. Example of informed legal consent for cryopreserving and storing semen from a minor (pre-**
14514 **pubertal boy/minor)**
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14521 # PART C – DEVELOPING APPLICATIONS

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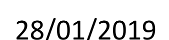


Chapter 29. Introduction of novel processes and clinical applications

29.1.Introduction

Advances in basic science, technology and medicine create opportunities for the development of novel tissue or cell graft-preparation processes (including changes to donor selection, procurement, processing, storage and distribution methodologies) or clinical applications. There are several key elements that must be observed to ensure the quality, safety and efficacy of novel processes and applications and, thus, the safety of donors and recipients:

a. Clinical need should be the predominant driver for the development of novel processes and applications for tissues and cells.
b. The involvement of, and close co-operation between, three groups – tissue establishments, clinicians representing organisations responsible for human application (ORHA) and Health Authorities – is essential to ensure that the principles of safeguarding quality, safety and efficacy are addressed. A clear structure identifying the responsibilities of each party, and how the different parties interact with each other, must be established and documented.
c. Comprehensive risk analysis should underpin the development and evaluation of novel processes and applications. This risk analysis should consider both the risks and potential clinical benefits of the novelty. Evaluation may comprise *in vitro*, *in vivo* and, where indicated, clinical follow-up studies according to the level of risk identified.

For established processes and applications, Part E of this Guide includes monographs appropriate to different processes and applications for tissues and cells. Monographs are useful tools for tissue establishments and Health Authorities, providing the minimum criteria to ensure the quality of different types of tissues and cells; they are tools that can be used by tissue establishments to design appropriate validation studies for new processes.

29.2. Regulatory considerations

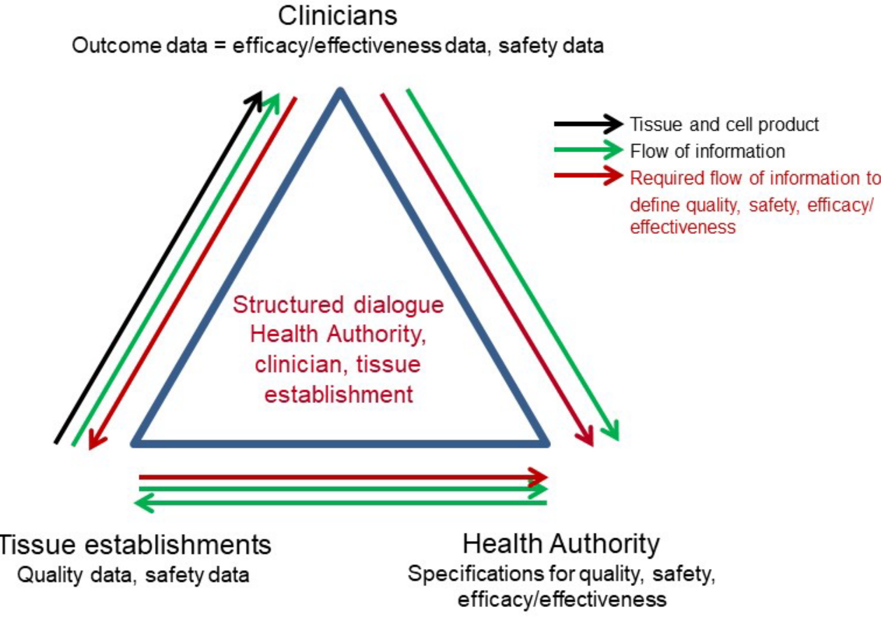
When a tissue establishment is developing a novel preparation process, or if it plans to provide tissues or cells for a new clinical application, it should consider whether the process or therapy might lead to a regulatory classification of the tissues or cells as medicinal products, or as advanced therapy medicinal products (ATMPs) or medical devices. If this is the case, the regulatory framework for the authorisation of the relevant product type will be applicable in the EU (for further information, see Chapter 30). However, if the starting material for the ATMP is a tissue or cell, the regulatory requirements regarding donation and traceability may have to be applied according to the EU Tissues and Cells directivess. Tissue establishments should engage with their Health Authority at an early stage of the product development cycle, in order to establish in advance which is the correct legal (regulatory) framework. This chapter addresses tissues and cells that are regulated under Directive 2004/23/EC in the EU.

This chapter is largely based on the guidance developed as part of the EU Joint Action VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation), which proposed regulatory principles for Health Authorities for the appraisal and approval of clinical evaluation protocols for blood, tissues and cells prepared with new processing methodologies [1]. These VISTART principles aim to guide stakeholders in the development and implementation of novel preparation processes or clinical applications, ensuring compliance with regulatory and technical

14564 requirements, and they propose an approach whereby the degree of risk associated with the novelty is
14565 linked to an appropriate clinical follow-up plan. This chapter has also built on the EuroGTP II project,
14566 which developed good-practice guidance for tissue establishments for the evaluation of the safety,
14567 quality and efficacy of tissue and cellular therapies and products [2].

14568 29.3. Interaction between key stakeholders

14569 When introducing novelties in the field of tissue and cells, three main stakeholders should interact
14570 closely. These are the tissue establishments, the clinicians in the ORHA and Health Authorities. They
14571 each contribute their specific expertise to establish a structured, safe and efficient approach to the
14572 introduction of new grafts and clinical applications. As consideration of the risks, both to the product
14573 effectiveness and to the safety of the patient are vital, consultation with all of these stakeholders is
14574 essential. There should be a formal agreement between the tissue establishment and the clinicians/ORHA
14575 clearly specifying their respective roles and responsibilities when setting up clinical evaluations. Figure
14576 29.1 describes schematically a structured flow of information between tissue establishment,
14577 clinicians/ORHA and Health Authorities.
14578



14579

14580 **Figure 29.1. Flow of information between tissue establishments, clinicians and Health Authorities**

14581 29.3.1. Responsibilities of the tissue establishment

14582 The tissue establishment is responsible for ensuring that the quality and safety of tissue and cell
14583 products meet the regulatory requirements and technical specifications necessary for release for clinical
14584 application. In this context, quality and safety result from the donor selection, the procurement of tissues
14585 and cells, the testing and the preparation processes. Preparation processes must be performed in
14586 compliance with the tissue and cell Good Practices Guidelines (Part D) and the EU Tissue and Cells
14587 directives requirements.
14588 Two different perspectives currently apply to the meaning of the term 'quality' within the field of
14589 tissue and cell product processing and both should be addressed by the set of quality-control parameters
14590 used to characterise the tissues and cells resulting from the preparation process: quality may be seen as
14591 the fulfilment of a specific set of standards, characteristics and requirements as predefined by the

preparation process, i.e. compliance of the tissue or cell product with its specifications (tissue and cell monographs). Quality may also be seen as an indicator of the safety and efficacy of the tissue or cell product. The critical parameters for novel tissue or cell preparation processes should cover both quality perspectives.

The safety profile of tissues and cells covers biological (infectious, immunological), physical (e.g. morphological appearance, integrity, elasticity) and/or chemical (e.g. toxicological, residual traces of reagents) influences. The safety of novel preparation processes or clinical application results from a careful and comprehensive risk analysis. Reference methodologies to perform a risk-based analysis of tissue and cells preparation processes and clinical applications are proposed by the EuroGTP II project (Good Practices for demonstrating safety and quality through recipient follow-up) [2], taking into account risks related to donor characteristics, procurement process and environment, preparation process and environment, reagents, reliability of microbiology testing, storage conditions, transport conditions, the presence of unwanted cellular material and the complexity of the preparation/application method.

Risk analysis is based on current evidence derived from known preparation processes, processing steps and products that are comparable to the tissues and cells obtained with novel preparation methods and/or new clinical applications. In circumstances where evidence is lacking, due to the grade of novelty and uncertainty, an analysis should be done to estimate the risks. The determination of risk levels – including a proposal for mitigation strategies – may be facilitated by supporting tools, such as the ones developed by the Euro GTP II Project, which provide a standardised methodology and an interactive assessment tool for risk analysis in the tissue, haematopoietic progenitor cell (HPC) and medically assisted reproduction (MAR) sectors.

Ultimately, the safety and efficacy of the tissue and cells product can only be confirmed by clinical outcome data. However, the tissue establishment should use *in vitro* and *in vivo* testing to investigate and mitigate any potential risks prior to clinical application.

29.3.2. Responsibilities of the clinicians/organisations responsible for human application

The perspective of the clinician focuses on product safety and efficacy in the context of clinical application of tissue and cell products. The clinician is responsible for obtaining appropriate patient consent for application, and for collecting clinical outcome data from patients. Clinical outcome data should be gathered from a well-defined patient cohort to demonstrate clinical efficacy and safety of the novelty.

The extent to which clinical outcome data are required to verify the safety and efficacy of novel tissues or cells depends on the level of risk of the novelty. A systematic analysis of literature evidence may be used to define the extent of clinical follow-up. In this case, the methodology used must be documented. Clinical follow-up studies should be designed to generate statistically significant data. The principles of Good Clinical Practice and the Declaration of Helsinki must be integral to the design and performance of clinical evaluation. The clinical outcome data must be shared between the clinician and the tissue establishment and forwarded to the Health Authorities by means of a clearly structured process. This is in addition to the routine biovigilance reporting procedures, which are mandatory.

If long-term clinical outcome data are required for the demonstration of clinical efficacy/effectiveness, then national, European or international registries might be a useful tool to obtain sufficient clinical information.

29.3.3. Responsibilities of the Health Authorities

Regulation of tissue and cells by independent bodies, e.g. Health Authorities, is important to ensure quality, safety, efficacy/effectiveness of tissue and cell products [1]. Regulation focuses on two key elements:

- data-driven, risk–benefit assessment of tissues and cells, based on well-established specifications ;

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14639 • risk-based decision-making on the approval of preparation processes or clinical applications by
14640 the Health Authorities.

14641 As novel tissue or cell products, inherent to the definition, have limited clinical data relating to
14642 quality, safety and efficacy, it can be challenging to assess their benefits and risks. Health Authorities
14643 can only approve tissue and cell products for routine clinical use based on sufficient data relating to
14644 safety and quality.
14645 In the case of innovative preparation methodologies or new applications, the normal authorisation
14646 procedure might need to be enhanced with associated clinical follow-up requirements, depending on the
14647 assessed risk. This approach will allow regulatory requirements to be balanced with timely access for
14648 patients to novel tissues and cell therapies and is in line with the new regulatory principles described by
14649 the VISTART Joint Action [1]. For example, limited authorisation of a preparation process might be
14650 issued with additional requirements – for example, that the tissue establishment and clinicians provide
14651 novel tissue and cell therapies for clinical application only to limited numbers of selected recipients,
14652 possibly in the context of an ethically approved clinical evaluation, or only to a limited number of named
14653 clinicians – so that the data needed to guarantee a comprehensive assessment of safety and quality can
14654 be systematically generated and reported by the tissue establishment to the Health Authority. At that
14655 point, if deemed appropriate, the Health Authority can issue a full approval. These regulatory approaches
14656 are being further explored in a new EU GAPP Joint Action [3] involving tissue and cell Health
14657 Authorities from multiple EU member states.
14658 A final proof of the quality, safety and efficacy of tissues and cells can only be provided by
14659 favourable clinical outcome data. Consequently, data resulting from clinical evaluation constitute the
14660 basis for a fully data-based, risk-dependent decision whether full approval of the novel preparation
14661 process or clinical application may be granted.
14662 Figure 29.2 summarises models of authorisation for novel preparation processes and clinical
14663 applications for tissues and cells.
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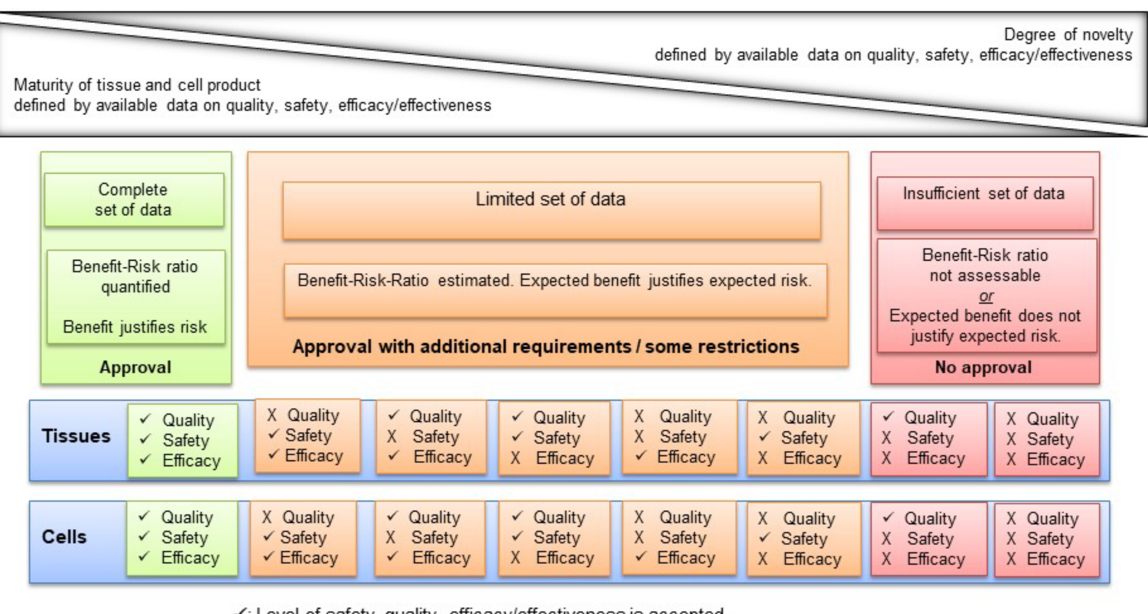

14666

14667 14668 **Figure 29.2. Regulatory models of risk assessment for novel preparation processes and clinical applications**

14669 14670

29.4. Life-cycle management of novelties and registries on consolidated practices

14671 Because novel tissues and cells are typically prepared, regulated and applied in the context of a
14672 continuously evolving 'state of the art', effective and efficient life-cycle management is of high
14673 importance. Life-cycle management comprises management of knowledge, change and uncertainty at
14674 the interface of the tissue establishment, clinician and the Health Authority. Thus, clinical follow-up of
14675 patients, i.e. the analysis of clinical outcome data and adverse reactions, is essential. Life-cycle
14676 management of tissues and cells also comprises a close regulatory oversight of the entire donation–
14677 distribution–application chain, requiring a close interaction between Health Authority, ORHA and tissue
14678 establishment.

14679 Life-cycle management of novel preparation processes or new clinical applications requires a
14680 broad and comprehensive data-based framework. Continuous review of preparation processes, clinical
14681 monitoring of recipients and availability of updated clinical information all contribute to product quality,
14682 safety and efficacy/efficiency profile. Such data-based frameworks require consideration of geographic,
14683 temporal and technical aspects: instead of local or regional overview of products, a much broader
14684 approach should be considered, utilising the tissue and cell monographs included in Part E of this Guide
14685 and by accessing to European databases, e.g. the European Cornea and Cell Transplantation Registry
14686 (ECCTR), the European Society for Blood and Marrow (ESBM) Transplant Patient Registry and
14687 European IVF Monitoring (EIM), whenever possible.

14688

29.5. References

14689 1. VISTART Joint Action (Vigilance and Inspection for the Safety of Transfusion Assisted Reproduction and
14690 Transplantation): Deliverable 5.4, "Principles for Competent Authorities for the evaluation and approval of

14691 clinical follow-up protocols for blood, tissues and cells prepared with newly developed and validated processing
14692 methodologies", available at https://vistart-ja.eu/home, accessed 4 January 2019.
14693 2. EuroGTP-II Project. Good Practices for demonstrating safety and quality through recipient follow-up, available at
14694 www.goodtissuepractices.eu/, accessed 4 January 2019.
14695 3. GAPP Joint action. Facilitating the Authorisation of Preparation Process for blood, tissues and cells, available at
14696 www.gapp-ja.eu, accessed 4 January 2019.
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Chapter 30. Developing cell technologies

30.1. Introduction

Therapies based on tissues and cells, such as those described in Part B of this Guide, are already well established in medical practice. In recent years, increasing numbers of tissue establishments have expanded their activities, providing starting materials or engaging in the preparation of more complex products based on human tissues and cells. This chapter provides an overview of this developing field and addresses technical considerations for professionals in tissue establishments who may be interested in learning about or developing such activities. An overview of these novel therapies involving the use of cells of human origin is presented in Figure 30.1.

It is important to note that, in different countries, these therapies may fall under different regulatory frameworks, including those for transplantation, medicinal products or medical devices. Irrespective of the content of this document, any operator active in the field should carefully consider the legal requirements that apply to the activities they are undertaking and it is advisable that, before starting any activities, they consult with the relevant authorities to understand the regulatory environment and seek any licence/authorisation that may be required.

In the European Union (EU), the use of cells falling under the definition of advanced therapy medicinal products (ATMP) is governed by specific requirements and procedures, including prior authorisation by the competent authority. An overview of the legal framework for the development of ATMPs in the EU is detailed in section 30.5 of this chapter.

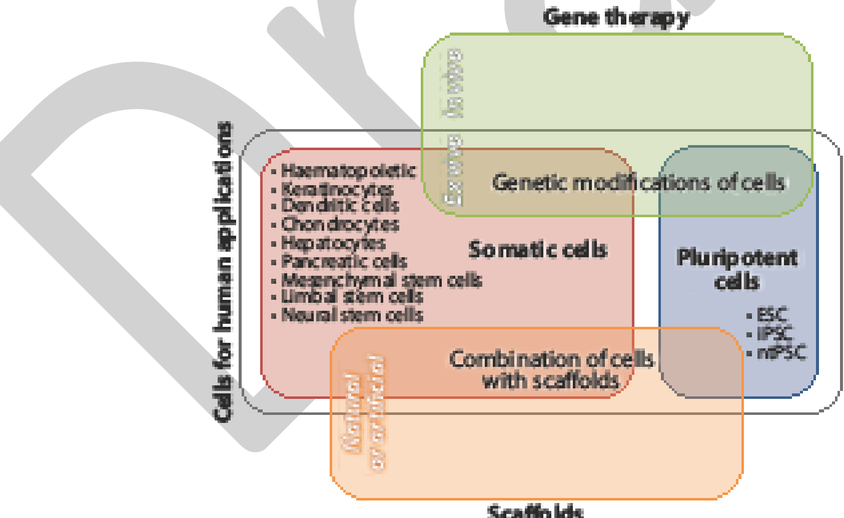

Figure 30.1. Novel therapies involving human cells

Note: ESC = embryonic stem cells; iPSC = induced pluripotent stem cells; ntPSC = nuclear-transfer pluripotent stem cells.

30.2. Cell types being used in developing new technologies

Many tissues are sources of different cell types that are currently being used or researched for the development of new therapeutic options. Due to their undifferentiated nature, stem cells can be expanded *in vitro* and differentiated into various tissue-specific cells. This unique characteristic can be exploited

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14726 to support the development of new therapies for the treatment of a number of conditions. However,
14727 before considering a new cell-based therapy, it is necessary to understand the physiological properties
14728 of each stem cell or progenitor cell type. In addition, in some countries the use of these stem cells may
14729 not be permitted, and this must be taken into consideration. Table 30.1 summarises some of the cell
14730 types currently used in developing novel cell-based therapies.
14731 The methods most commonly used to obtain pluripotent stem cells (PSC) are shown in Figure
14732 30.2. Haematopoietic progenitor cells (HPC) and multipotent and lineage progenitor cells are discussed
14733 in further detail in Chapters 22 and 32 respectively.
14734

14735 **Table 30.1. Some cell types being used to develop novel cell-based therapies**

	Cell type	*Source*	*Processing*
Pluripotent	Embryonic stem cells (ESC)	Obtained from the inner cell mass of blastocyst which have been cryopreserved and are no longer to be used for fertility treatment	Cultured embryos (maximum 14 days) are dissociated and the inner cell mass is removed and cultured for a few more days. Then inner cell mass outgrowths consisting of potential ESC are isolated and expanded to create stem cell lines.
	Reprogrammed stem cells	Somatic cells which are reprogrammed to an embryonic stem-cell-like state	Reprogramming is achieved by introducing into adult cells (e.g. epithelial cells) a defined and limited set of transcription factors (e.g. stemness transcription factors) giving rise to induced pluripotent stem cells (iPSC). Cell reprogramming can also be achieved by nuclear transfer, giving rise to nuclear-transfer pluripotent stem cells (ntPSC).
Multipotent (lineage-restricted)	Somatic stem cells	Found in various tissues and may be isolated from extra-embryonic tissues, foetal specimens and adult tissues – generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, neural stem cell etc.)	Specific protocols have been developed, depending on the cell type. For details see Chapter 32.
Lineage-committed	Progenitor cells, e.g. haematopoietic progenitor cells	Somatic cells that are committed to a specific mature cell fate and can divide only a limited number of times	Can be transplanted without *in vitro* expansion (e.g. bone marrow, peripheral blood, cord blood), or cultured *in vitro* and differentiated into more restricted cell types for clinical application.

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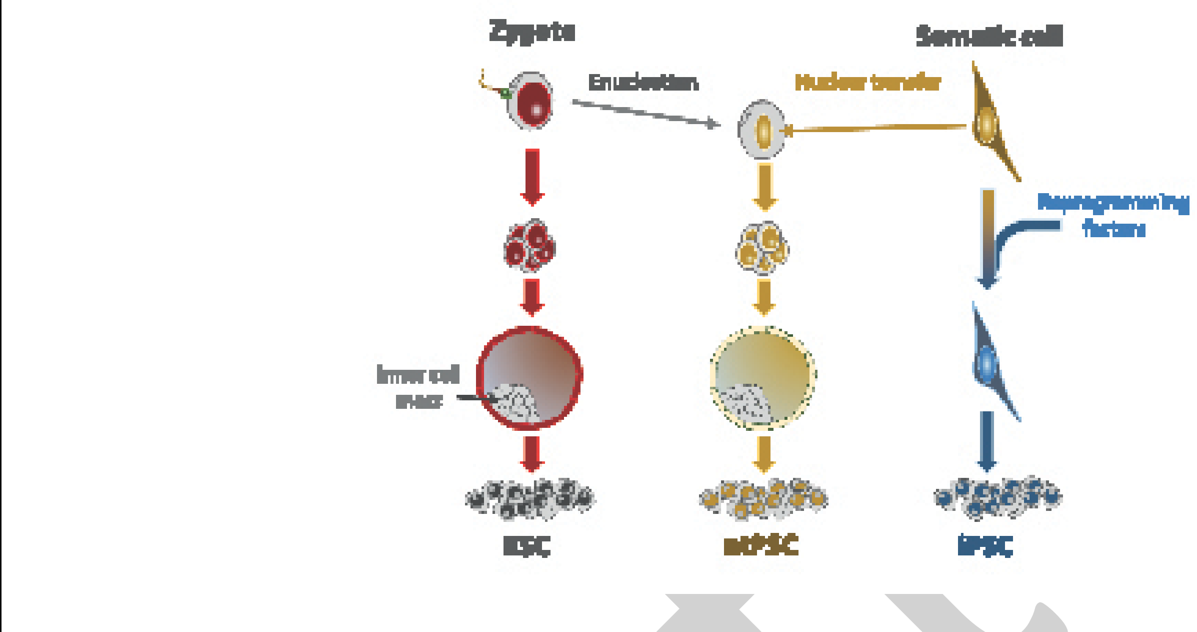

Figure 30.2. The most common methods used to obtain pluripotent stem cells

Note: To obtain embryonic PSC (ESC), embryos are developed until the blastocyst stage, when the inner cell mass is isolated and cultured to derivate the cells. Zygote can be enucleated, and a somatic cell nucleus is then transferred into it. The nuclear-transfer zygote is then developed until the blastocyst stage, and the inner cell mass is isolated and cultured to obtain the nuclear-transfer PSC (ntPSC). Somatic cells can also be reprogrammed into induced PSCs (iPSC) by using a transcription factor cocktail.

30.3. Processing cells for human application

In the preparation of cells for human application, different levels of processing may be required in order to deliver cells with the required characteristics and functionality. Some cells can be transplanted without *in vitro* expansion (e.g. HPC procured from bone marrow, HPC procured from peripheral blood, HPC procured from cord blood, hepatocytes, beta cells), whereas others need to be cultured *in vitro* and differentiated into more restricted cell types and, finally, some others undergo many manipulations to render the final product suitable for clinical application. In this section some of the most commonly used processing methods are discussed.

In the EU, many of the following processing methods are considered as substantial manipulations and thus the resulting products are governed as ATMP under the medicines rules, which include specific requirements that must be respected when the activity is undertaken in the EU or if the resulting cells are intended to be used in the EU (see section 30.5 for further details).

30.3.1. Culturing cells

The origin and procurement of the starting material to isolate cells for therapeutic use is considered critical for the yield and identity/purity of the final cell population. Processing steps – such as derivation and/or expansion of cells, addition of cryoprotectants and all steps involved in the culture of cells – must be undertaken in an environment that is fully compliant with good manufacturing practices (GMP). The initial procurement of tissue or cells from a human should always follow the regulations and guidelines related to human tissue/cell donation, procurement and testing, and universal precautions must be applied to minimise the risks of contamination, infection and pathogen transmission (see Chapters 4, 5 and 6).

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Culture conditions are critical during expansion and differentiation of cells in culture [1]. The culture conditions for a specific cell type should be defined to control the number of cellular duplications and to achieve an adequate balance between number of passages and duplications. Antibiotics are not usually included in cell-culture media because they may mask the presence of a low level of bacterial contamination and, therefore, have the potential to cause infections in some recipients. Microbiological testing is required when culturing cells, which should be done at different critical steps and before cell release (see Chapter 10).

Validation of the preparation process should be carried out with respect to maintaining genetic stability and the relevant biological properties, as well as avoiding malignant transformation.

The impact of raw materials of biological origin used in the production of cells for therapeutic use on the quality, safety and efficacy of the cells has to be evaluated by risk assessment. Guidance is provided by the *European Pharmacopoeia (Ph. Eur.)* [2]. A safety issue when culturing human cells is the use of materials of animal origin, such as media or growth factors. The use of this xeno-material should be avoided as much as possible by using human-derived factors, for example by obtaining serum from the intended recipient. If animal materials cannot be avoided, specification and verification of both source and method of preparation of the material is required (for example, guidance for bovine serum can be found in *Ph. Eur.* monograph 2262). Culture media and other reagents derived from animals must be evaluated for the risk of contamination with micro-organisms, particularly viruses and transmissible agents of human pathologies such as transmissible spongiform encephalopathies (TSE) (*Ph. Eur.* 5.2.8).

Documentation that demonstrates the application of appropriate quality-assurance measures by suppliers of media of animal origin, including origins and veterinary certificates for the animals used in the preparation of the material (e.g. bovine serum albumin), must be obtained. Certificates must be supported by audit trails for collection, pooling, shipping and final formulation by the third-party supplier. The use of raw materials and processing materials that are supplied with a TSE certificate from the European Directorate for the Quality of Medicines & HealthCare (EDQM) minimises the risks of infection from TSE [3]. The requirements for sourcing/donation, procurement and testing are set out in Annex 2 of the GMP Guidelines and in the guideline on xenogeneic cell-based medicinal products. For further guidance on cell culture, refer to the report (by the second Task Force of the European Centre for the Validation of Alternative Methods) on Good Cell Culture Practice [4].

At the point where the cells will not be expanded any further, the downstream process begins. This includes the final collection and subsequent process steps of concentration or volume reduction of the collected cells, washing or clarification of the collected cells, formulation of the cells into an appropriate medium for preservation and then filling their final container with the formulated cells for cryopreservation and storage, or for direct delivery to patients. When a cell-based product is delivered for use, it must be shipped under appropriate conditions to the clinical site, prepared for application to the patient and then administered by a medical doctor or trained healthcare professional.

30.3.1.1. Microbiological testing when culturing cells

Culturing cells includes a broad range of procedures that differ, depending on the cell type, in many aspects such as source material, finished product, culture reagents, time in culture, expansion, differentiation, quality-control analysis and storage procedures. All these differences make it difficult to establish a general rule for microbiological testing. Therefore, for each procedure, Quality Risk Management (QRM) should be applied to determine the quality-control strategy to be followed through the whole process and to identify critical steps to reduce the possibility of contamination and cross-contamination.

As a general recommendation, both the starting material and the finished product should be tested. For the starting material, microbiological tests should be carried out on the cells (or the cell source if the cells of interest are in too low numbers) and the culture reagents to ensure the absence of bacterial, fungal or mycoplasma contaminations. It is recommended to use the methodology described in the *Ph. Eur*. If other methods are used, they must always be validated in advance.

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14807 When the starting material, including the cells themselves, cannot be stored in quarantine until
14808 the results of the microbiological analysis are obtained (for reasons of cell instability), the processing
14809 steps can start without the results being available. However, it would be necessary to study the potential
14810 risks of using this 'non-tested material' and document it following the principles described in the QRM
14811 protocol.
14812 In principle, the microbiological analysis should be done either in intermediate-cell products or
14813 finished product. However, when the number of cells is scarce, it may be acceptable to perform the
14814 analysis with samples of washing media, supernatants or cell-culture media. If any microbiological
14815 contamination is detected, it is necessary to identify the strain and to investigate its source. In this case
14816 the product should not be used for clinical application unless a risk–benefit analysis indicates that it is
14817 the best option for the patient.
14818 The finished product should be analysed for its microbiological quality before it can be released.
14819 Absence of bacteria, fungi and mycoplasma should be demonstrated. However, in specific cases when
14820 this approach is not feasible, the release of the finished product before the microbiological results are
14821 available may be justified. In this context, implementation and documentation of adequate processing –
14822 processing that provides sufficient assurance of the microbiological quality of the product when released
14823 – is essential. This will include in-process microbiological tests that have been established on the basis
14824 of risk analysis, usually including sterility testing of the culture media and of samples from the
14825 intermediate product at critical steps. It is recommended to use tests as described in *Ph. Eur.* 2.6.1 and
14826 2.6.27 [5], the results of which are available in 7-14 days, depending on the method used. Alternative
14827 methods have been developed in recent years, and some of these methods have shown potential for real-
14828 time or near real-time results (*Ph. Eur*. General Monograph 5.1.6). Absence of mycoplasma should also
14829 be analysed at given critical steps (*Ph. Eur*. 2.6.7) and endotoxin determination may also be
14830 recommended (*Ph. Eur.* 2.6.14 and *Ph. Eur.* 5.1.10). More extensive details on microbiological testing
14831 may be found in Chapter 10.
14832 When cells are allogeneic it is recommended to include the analysis of adventitious viruses, both
14833 in cell source material and in the finished product, in order to avoid transmission to the patient (see *Ph.*
14834 *Eur.* 5.1.7 for viral safety). Adventitious viruses can be analysed by different methodologies but
14835 polymerase chain reaction (PCR) assays are the most commonly used (indications for validation
14836 described in *Ph. Eur.* 2.6.21). In order to establish the virus that should be tested, it will also be necessary
14837 to do risk analysis [6]. Some examples of adventitious viruses that can be included are: *adeno*-associated
14838 virus, *Adenovirus*, *Bunyavirus*, *Cytomegalovirus*, Epstein–Barr virus, *Flavivirus*, hepatitis A virus,
14839 hepatitis B virus, hepatitis C virus, human *Herpes* virus 1 (HHV1), HHV6, HHV7, HHV8, human
14840 immunodeficiency virus types 1 and 2 (HIV-1, HIV-2), human *papilloma* virus, human *rotavirus* (HRV),
14841 human T-cell leukaemia virus type-1 (HTLV-1), HTLV-2, influenza, measles, Norwalk virus, *parvovirus*
14842 B19 (Parvo-B19), rubella and *enterovirus* (polio).

14843 *30.3.1.2. Considerations for quality control of other parameters for cultured cells*

14844 It is important to demonstrate that the cell-based product meets specifications before release. For
14845 this reason, quality controls should include not only cell viability (*Ph. Eur.* 2.7.29) and genomic studies,
14846 but also cell identity, testing of biological activity, and other microbiological controls. When possible,
14847 fluorescence-activated cell sorting (FACS) to analyse the presence or absence of specific cell-surface
14848 antigens (CD) is the best method to quantify the purity of the culture (*Ph. Eur.* 2.7.24). For example,
14849 haematopoietic progenitor cells should be positive for CD34 (*Ph. Eur.* 2.7.23) whereas mesenchymal
14850 stem cells (see Chapter 32 for nomenclature clarification) are negative for the antigen CD45 but positive
14851 for CD105, CD73 and CD90 (see Chapter 32 for specific information on cell-surface antigens of several
14852 somatic stem cells). However, in many cases the short time from cell culture to release and application
14853 is challenging, and these tests must be performed during processing.
14854 The selection of appropriate markers is fundamental in the standardisation of isolation conditions
14855 and to identify cell populations, heterogeneity and yield. However, in many cases there are no known
14856 specific surface antigens for stem cells, which makes their purification difficult.

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14857 Control of genomic stability is also critical before releasing the cells for transplant in humans.
14858 Telomerase activity, proliferative capacity and senescence are also quality controls of relevance for
14859 human pluripotent stem cells.

14860 *30.3.1.3. Master cell banks, working cell banks and cell stock*
14861 Some cells, including stem cells, are used to produce master cell banks, which are then banked
14862 for future use as starting materials to manufacture cell-therapy products. A cell line is established from
14863 a single clone and this cell line is used to make up the master cell bank. This master cell bank must be
14864 characterised and extensively tested for contaminants such as bacteria, fungi and mycoplasmas. In
14865 addition, sterility and endotoxin testing, as well as PCR testing for viruses, may be required.
14866 Cell lines used for patient treatment are placed in cryovials, and cryoprotectants are added before
14867 the cells are frozen and stored in the vapour phase of liquid nitrogen. The use of cryoprotectants and the
14868 control of cooling and warming rates during freezing and thawing are essential to minimise cell death
14869 and to increase cell attachment after thawing. This is of special importance during cryopreservation of
14870 human pluripotent stem cells [7]. The temperature inside the liquid nitrogen tank should be continuously
14871 monitored in order to ensure that the cells are stored under stable conditions.
14872 It is recommended that master cell banks are stored in two or more widely separated areas within
14873 a production facility, and also at a distant site, in order to avoid loss of the cell line. Dual-site storage of
14874 all cells is accomplished with on-site storage and off-site storage.
14875 Quality controls should be the same as described above for cell release and should be carried out
14876 before cryopreservation and after thawing to ensure that cell proliferation and viability, genome stability
14877 and purity of the culture have not been affected by the freezing process.
14878 The working cell bank is a pool of expanded well-characterised cells derived from the master cell
14879 bank. The working cell bank is prepared from a single homogeneously mixed pool of cells. One or more
14880 of the working cell bank containers is used for each cell expansion. The characterisation and quality
14881 controls carried out for the working cell bank after cell thawing must be exactly the same as is done for
14882 the master cell bank, and the same specifications should be maintained.
14883 Where cell-based products are generated from a cell stock obtained from a limited number of
14884 passages, and the stock does not cover the total life-cycle of the ATMP, it may be necessary to collect
14885 extra cells from new donors. The impact of these changes should be assessed and validated. It is
14886 recommended that cell stocks be handled in accordance with the principles outlined above for cell banks
14887 in regard to handling, storage and release.
14888 Establishment of new cell banks/new cells stocks should be done in accordance with GMP. In
14889 exceptional and justified cases, the use of cell stocks/cell banks that were generated prior to the entry
14890 into force of Regulation 1394/2007 without full GMP compliance may be acceptable. In these cases, a
14891 risk analysis should be conducted to identify the testing requirements necessary to ensure the quality of
14892 the starting material; within the EU, approval from the competent authorities prior to use should be
14893 obtained [8].

14894 **30.3.2. Genetic modifications of cells**
14895 The genetic engineering of human, animal, plant and microbial cells in the laboratory became
14896 established biotechnology practice in the latter part of the 20th century. Techniques have been developed
14897 to allow the addition, removal and editing of gene sequences within cells, with ever greater precision,
14898 and the reliable expression of the products from such modified genes *in vitro* and *in vivo*. New
14899 therapeutic products, such as monoclonal antibodies, have been manufactured using such genetically
14900 modified cells grown *ex vivo* in bioreactors.
14901 There is now much interest in using the techniques developed for *in vitro* genetic modification of
14902 cells for direct therapeutic use *in vivo* for the treatment of a variety of diseases. This area of medicine is
14903 known as gene therapy. Modern gene sequencing has allowed specific genes associated with certain
14904 diseases to be identified and new techniques allow those genes to be synthesised and modified in the

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14905 laboratory. In order to have a therapeutic effect, the gene needs to gain access to the target cells and be
14906 appropriately processed within the cell. This is achieved using a gene carrier or vector.
14907 In the last decade, gene therapy has been a fast-moving area, and a number of issues related to the
14908 quality requirements of these complex, patient-specific therapies are arising as the field is advancing
14909 [9].

14910 *30.3.2.1. General considerations in gene vectors for genetic modification of human cells*

14911 The starting point for most gene vectors is a synthetic DNA plasmid. This contains the therapeutic
14912 gene sequence flanked by suitable sequences to allow its appropriate replication and expression in the
14913 target cell and by further sequences which allow the manufacture of multiple copies of the therapeutic
14914 gene in laboratory cell culture in order to have sufficient to transfect the target cells. Plasmids are
14915 relatively small, circular forms of DNA and several techniques have been developed that enable these
14916 plasmids to gain entry to cells *in vitro*. Most are engineered to allow multiple copies to be manufactured
14917 in bacterial cell culture, then extracted and purified before being used for gene therapy.
14918 Techniques for transfecting cells with plasmids cannot generally be used *in vivo*, although muscle
14919 cells have been induced to take up plasmids following intramuscular injection and respiratory tract
14920 epithelia using liposomal delivery, with transient expression of the therapeutic genes. They are therefore
14921 more commonly used to transfect human cells *ex vivo* as a means of genetic manipulation before the
14922 cells are used therapeutically. For example, plasmids have been used as suitable vectors for delivering
14923 the genes to *in vitro* somatic cell cultures required to derive iPSC (see §30.2).
14924 However, because of low transfection efficiency and generally transient nature in human cells,
14925 plasmids are not the vector of choice for *ex vivo* genetic manipulation of human cells or *in vivo* gene
14926 therapy. Instead, they are used to manufacture synthetic viral gene vectors in laboratory cultures of
14927 human or animal cell lines. Two or more plasmids are usually used to transfect the cell line. The plasmids
14928 contain not only the therapeutic gene and required flanking sequences, but also genes coding for
14929 important viral proteins, so that multiple viral particles will be generated with the therapeutic genes
14930 appropriately packaged within each particle for delivery to the target cell.
14931 Viral vectors are based on naturally occurring viruses, selected for their particular characteristics,
14932 such as whether they selectively transfect certain tissue types and whether they integrate their genes into
14933 the host cell chromosome, or express them extra chromosomally. Specific packaging cell lines have been
14934 produced that allow the necessary replication of the vector in culture, but do not allow viral replication
14935 genes to be packaged in the viral particles produced, rendering the vector replication non-competent. A
14936 number of viruses have been used as the basis for human gene therapy, including *retrovirus*, *Adenovirus*,
14937 *lentivirus*, *Herpes simplex* virus, *vaccinia*, pox virus and *adeno*-associated virus.
14938 Because of the potential risk of harmful infection using viral gene vectors and the unintended
14939 side-effects of genetic manipulation, tissue and cell establishments must consult appropriate experts in
14940 designing or selecting a vector for use in genetic manipulation of cells for human application. It is also
14941 essential that the vector is manufactured under highly controlled conditions, with rigorous quality
14942 control [10, 11]. Establishments should use experienced, specialist GMP manufacturers for this purpose.

14943 *30.3.2.2. Post-genetic modification processes*

14944 Extensive characterisation of the genetically modified cells must be performed, including
14945 establishing the number and location of integration events, sequencing of integrated sequences to
14946 establish the integrity of the molecular construct, removal of the transgene (if needed), investigation of
14947 the possibility of vector replication and viral reactivation, and confirmation of the genetic stability of
14948 the cells [12].
14949 When the transgene is not intended to modify the cells or induce differentiation, a large number
14950 of experiments should be performed in order to demonstrate that modified cells have the same
14951 morphology/phenotype, genetic profile and functionality as the (pre-modification) parental cells.
14952 Unexpected changes in cell morphology, function and behaviour should be at least investigated and

14953 documented, and (depending on the magnitude of the changes) genetically modified cells must be
14954 discarded.
14955 If the genetically modified cells are intended to have replacing activity, chromosomal integrity
14956 should be shown. Similarly, when genetic modification is performed to induce differentiation, the
14957 efficacy of such a process must be characterised and documented. When using pluripotent cells, any
14958 undifferentiated cells should be removed or killed to avoid teratoma formation after grafting. The purity
14959 criteria should be established and be within determined limits.
14960 When genetic modification is performed for *ex vivo* production of secreted proteins of interest,
14961 pharmacokinetic studies should be designed in order to address not only expression, distribution and
14962 persistence of the transgene, but also dosage of protein release per cell and stability under *in vitro* and
14963 *in vivo* conditions. Toxicological studies should also be performed in order to avoid any unexpected
14964 effects. Similarly, when the cells that produce the gene product are encapsulated in biocompatible
14965 material, the appropriate secretion activity and potential toxicity should also be characterised and
14966 reported.

14967 *30.3.2.3. Transient expression*

14968 When transient genetic modification is intended, for example to induce cell differentiation, all
14969 genetic constructs must be removed from the final cell product. For this purpose, it is important to design
14970 not only the plasmid sequence but also the molecular strategy to verify that any traces of the plasmid
14971 used have been removed, to avoid future expression of genes or aberrant constructs.

14972 *30.3.2.4. Purity and cell selection*

14973 The purity of genetically modified cells is related to the efficacy of the transfection/transduction
14974 method used. In addition, when choosing the genetic modification method to be used, a selection marker
14975 can be introduced to increase the purity and consistency of the gene delivery method. The selection
14976 method, such as fluorescent or magnetic, should be chosen on the basis of the intended use. Cell selection
14977 is an important step to separate genetically modified cells from those that were not successfully
14978 modified. A complete description and a system of monitoring of the method used for the selection and/or
14979 purification is mandatory. The consistency of the method must also be demonstrated in different cell
14980 preparations.
14981 The homogeneity and genetic stability of the modified cells should be characterised, including
14982 ascertaining that all cells in the purified population contain the intended genetic modification. The
14983 testing methods used for this should be cell type and vector-specific as necessary.
14984 Furthermore, any observable change in morphology, function or behaviour of the purified cell
14985 population – whether caused by the genetic modification, the process of genetic modification or the
14986 purification process – should be documented. Special attention should be paid to the proliferation and
14987 differentiation properties of the modified cells and how they compare to the original unmodified cells.

14988 *30.3.2.5. Cell banking of genetically modified cells*

14989 After genetic modification of the cells, they can be cultured, selected and/or frozen. When
14990 expression of the gene is permanent, the production of a master cell bank (see §30.3.1.3) with modified
14991 cells is recommended. The master cell bank will give the opportunity to make the appropriate quality
14992 controls without excessive passaging of the cells.
14993 A complete description of the post-modification steps should also be registered and appropriately
14994 monitored.

14995 *30.3.2.6. Dosage*

14996 It is critical to select the appropriate dose of the product when the cells are used to secrete a
14997 functional protein to produce a paracrine effect. The final dose will depend on several parameters,
14998 including the level of expression, the number of vector copies per cell and the number of cells grafted.
14999 Where possible, the number of grafted cells should be adjusted to administrate the desired protein dose.
15000 Pre-clinical studies may help to set the correct dose, but the results obtained in animal models cannot
15001 always be extrapolated to human beings since the expression of the gene may change depending on the

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niche of the transplanted area. For this reason, applying a single dose in clinical trials is not feasible in most cases. Thus, applying at least two doses, the minimal effective dose and the maximum tolerable, will provide important information for future clinical trials.

30.3.3. Tissue decellularisation and combination of cells with natural scaffolds

Decellularisation of donated tissues is a technique commonly performed in tissue establishments with the purpose of producing a cellular-neutralised parenchyma that may have several uses. These extracellular matrices (ECM), also known as ‘scaffolds’, may in some instances be used directly for human application (e.g. heart valves, large vessels or dermal matrices) providing structural benefits while reducing immunological rejection and the risk of contamination. Alternatively, cells can be combined with these ECM and used in patients to improve or replace biological tissues.

Decellularisation and combination of ECM with cells are addressed in more detail in Chapter 31.

30.4. Safety considerations when applying cells to patients

Many of the early clinical successes using intravenous infusions of cell-based products have been seen subsequently in the treatment of systemic diseases such as graft *versus* host disease and sepsis. However, it is becoming more accepted that diseases involving peripheral tissues, such as cartilage repair, may be better treated with methods that increase the local concentration of cells. Direct injection or placement of cells into a site for tissue repair may be the preferred method of treatment, as vascular delivery suffers from a ‘pulmonary first-pass effect’, where intravenous injected cells are sequestered in the lungs.

Cells may suffer substantial functional changes resulting not only from culturing, *ex vivo* activation or genetic manipulation, but also as consequence of their human application. When cells are transplanted, the environment changes considerably and these changes can modify the morphological and functional characteristics of the cells; therefore, evaluation of tumourigenicity should also be integrated when cells are implanted into the patient. Short-term and long-term post-grafting follow-up of each patient is critical in autologous and allogeneic applications. Details of vigilance requirements of both recipient and donor of tissues and cells are addressed in Chapter 16. Pharmacovigilance requirements in the EU are defined in Regulation EU 1235/2010 and Directive 2010/84/EU, and specific guidance for ATMP issued by the European Medicines Agency is available for consultation [13].

Cells are not classical drugs and need specific requirements when manipulated. It is important that the administering professionals have some basic knowledge about cells. Some clinical trials failed because of a lack of training of the professionals who manipulated the cells before and during human/clinical application. Detailed instructions should be given that include cell manipulation and tissue pre-treatment, to avoid cell death or modification of the biological properties of the cells. Key requirements include having adequate pre-clinical data, independent oversight and peer review, fair subject selection, informed consent, research subject monitoring, auditing of study conduct, and trial registration and reporting

30.5. Legal framework for the development of advanced therapy medicinal products in the European Union

To provide a common framework for the marketing of ATMP in the EU, of the European Parliament and of the Council on advanced therapy medicinal products (hereafter ‘the ATMP Regulation’) was adopted in 2007. Specifically, cells used in human application that have been subject to substantial manipulation, and/or cells that are used for an essential function or functions in the recipient different from their function in the donor, are regulated as medicinal products in the EU.

According to Article 1 (a) of the ATMP Regulation, an ATMP is any of the following medicinal products for human use:

a. a gene therapy medicinal product;

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b. a somatic cell therapy medicinal product;
c. a tissue-engineered product.

A gene therapy medicinal product is a biological medicinal product that fulfils these two characteristics:

a. it contains an active substance that contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;
b. its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products must not include vaccines against infectious diseases.

A somatic cell therapy medicinal product is a biological medicinal product that has two characteristics:

a. it contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor;
b. it is presented as having properties for (or is used in humans with a view to) treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its tissues or cells.

For the purposes of point (a), the manipulations listed in Annex I to Regulation 1394/2007/EC are not considered as substantial: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing, cryopreservation and vitrification. Note that this list is non-exhaustive. Thus, based on scientific considerations, other manipulations may also be judged 'non-substantial' (e.g. manipulations that have been used in clinical practice in a hospital setting over many years).

A tissue-engineered product is a product that:

a. contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor;
b. is presented as having properties for (or it is used in humans with a view to) regenerating, repairing or replacing human tissue.

A tissue-engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, bio-molecules, biomaterials, chemical substances, scaffolds or matrices. Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, are excluded from this definition.

The cornerstone of the ATMP Regulation is that a marketing authorisation must be obtained before the marketing of ATMP. In turn, the marketing authorisation can only be granted if, after a scientific assessment of the product's quality, efficacy and safety profile, it is demonstrated that the benefits outweigh the risks. The Committee for Advanced Therapies (CAT) is a specialised and multidisciplinary committee at the European Medicines Agency (EMA) responsible for assessing the quality, safety and efficacy of ATMP that follow the centralised procedure for marketing authorisation and it assists in the preparation of any documents related to fulfilment of the objectives of Regulation 1394/2007, where relevant. The application for a marketing authorisation must be submitted to the EMA and the final decision is taken by the European Commission.

Developers of products based on genes, tissues or cells can seek advice from the CAT on whether a specific product is an ATMP. The procedure is intended as an incentive for developers, who can

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15094 ascertain at an early stage of development if their product must comply with the requirements that apply
15095 to ATMP.
15096 Where ATMP contain human cells or tissues, Directive 2004/23/EC applies to the donation,
15097 procurement and testing of the tissues and cells.
15098 The ATMP Regulation gives member states the power to authorise the use of custom-made ATMP
15099 prepared on a non-routine basis in the absence of a centralised marketing authorisation, provided that
15100 the product is used for individual patients in a hospital and under the professional responsibility of a
15101 medical practitioner. This 'hospital exemption' requires the application of national requirements on
15102 quality, traceability and pharmacovigilance equivalent to those required for authorised medicinal
15103 products.
15104 It is important to stress that, in the EU, ATMP are medicinal products. It follows that the overall
15105 regulatory framework governing medicines (including, but not limited to, rules on manufacture,
15106 distribution, packaging, labelling, evaluation of risks and benefits, determination of the data needed to
15107 demonstrate efficacy and safety, pharmacovigilance and advertising of medicines) apply to ATMP.
15108 Furthermore, use of ATMP in an investigational setting is also subject to EU rules on clinical trials.
15109 However, flexibility in the development of ATMPs is important to anticipate the rapid evolution
15110 of science and technology in the field. To facilitate the process, the EU introduced the risk-based
15111 approach [14]. The purpose of the risk-based approach is to obtain a profile of the risks associated with
15112 the use of a specific ATMP by identifying the various risks associated with the clinical use and the risk
15113 factors inherent to the ATMP with respect to quality, safety and efficacy. The approach aims to provide
15114 the possibility of moving away from guideline-based ATMP development and to facilitate science-driven
15115 development strategies of ATMPs.
15116 Additional information about the EU regulatory framework for ATMP can be found at the EMA
15117 and EC Internet websites [15].
15118 It is important to emphasise that, in Part C of this Guide, the term 'cells' or 'cell therapy' can be
15119 used to refer to situations that may be regulated as ATMP in the EU. This guidance is not intended to
15120 affect the scope of the EU rules on medicines, and any operator who intends to process, store, distribute
15121 or use cells in humans should first seek advice from national authorities on the appropriate, applicable
15122 legal framework.

15123 **30.5.1. National competent authorities**

15124 In EU member states, human cells for human application can be subject to different regulatory
15125 frameworks (depending on the intended use, mode of action and degree of manipulation). Advice on the
15126 classification of a specific cell therapy can be sought from the national competent authorities or from
15127 the CAT [16]. In some countries, the regulatory body is the same for all cell therapy products whereas,
15128 in others, those classified as ATMP are regulated by a different body/agency from those classified as
15129 cells for transplantation.
15130 The ethical and legal position on the use of human stem cells or progenitor cells, as well as the
15131 regulatory oversight, differs in countries around the world. In the EU, each member state is able to make
15132 decisions on the use of progenitor stem cells for basic research. However, they must be compliant with
15133 the requirements stated in the ATMP Regulation if they are to be used for the manufacture of ATMP for
15134 treating patients.
15135 Some countries have national legislation on paediatric donors that should be taken into account
15136 when cells from children are considered.

15137 **30.5.2. Independent ethics committees**

15138 Local/regional/national Independent Ethics Committees (IECs) are important bodies designated
15139 to approve and review biomedical and behavioural research involving humans, including the scientific
15140 rationale for the clinical application of a new therapy. For the latter, IECs should consider the irreversible
15141 nature of some gene/cell therapies and address the acceptability of exposing a donor to a research

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15142 protocol for the benefit of the recipient, in particular where the donor is a relative of the subject,
15143 especially a parent or a sibling, to be included in the trial. IECs should also check appropriate traceability
15144 and guarantees regarding subject data protection and confidentiality. Written informed consent for
15145 receiving a cellular therapy is considered a prerequisite, as in any clinical trial.
15146 The International Society for Stem Cell Research (ISSCR) guidelines recommend that special
15147 emphasis be placed on the risks of stem cell-based clinical research during the informed consent process.
15148 The risks include tumour formation, immunological reactions, unexpected behaviour of cells, unknown
15149 long-term health effects and sensitivities around the source of cellular products [17].

15150 **30.5.3. Considerations on conduct of clinical research**

15151 Clinical research, including trials of experimental interventions, is essential in translating cell-
15152 based treatments and it requires the participation of human subjects, whose rights and welfare must be
15153 protected [17]. All people involved, including sponsors, investigators, oversight bodies and regulators,
15154 must be responsible for ensuring the ethical conduct of clinical trials. As with all clinical research,
15155 clinical trials of stem cell-based interventions must follow internationally accepted principles governing
15156 the ethical design and conduct of clinical research and the protection of human subjects [18, 19]. In the
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15221 implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
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Chapter 31. Preparation of natural scaffolds

31.1 Introduction

The state of the art on tissue manipulation is in constant growth and evolution, due to the diversity of its clinical applications. Decellularisation of tissues is a reality, as this process is currently being applied in clinical practice with different type of tissues (details related with technical procedures can be found in Chapter 8 and Appendix 33). Despite this, decellularisation processes are continuously improving and adapting in order to obtain new tissues and/or clinical applications.

This chapter aims to provide a general overview of the potential new uses of scaffolds obtained through different decellularisation processes, addressing its main advantages and challenges. Moreover, this chapter present a general overview of the challenging process of bioprinting, which is now in a period of exponential growth, with no consolidated application yet.

Decellularisation of substances of human origin (SoHO) must maintain an equilibrium between eliminating cellular content and maintaining the mechanical and biological properties of the extracellular matrix (ECM). It is challenging to develop a decellularisation process that fully retains the essential properties for its final clinical applications. In this context, a variety of investigation lines are currently open with the aim to improve the properties of the SoHO and give solutions to medical conditions that at the moment do not have an appropriate therapy, or where improvements are still needed.

The major goal of a scaffold is to create the *in vivo* micro-environment, which is mainly provided by the ECM. Apart from the particular criteria needed for specific applications, an ideal scaffold designed for clinical use should fulfill a set of technical requirements. Firstly, biocompatibility and biodegradability are required, to allow scaffold replacement by proteins synthesised and secreted by native or implanted cells [1-3]. However, some applications require a compromise between complete degradation and mechanical support; biomaterials have been successfully used to constrain the post-myocardial-infarction failing heart, preventing it from further remodelling and dilatation [4-5]. The degradation products must be non-toxic and readily removed from the body. Additionally, the material must be clinically compliant, which means using Good Manufacturing Practices (GMP) to minimise inflammatory and immunological response, avoiding further tissue damage [3]. Moreover, since cell-degradation products are toxic to other cells, it would be important for the scaffold to allow host macrophages to infiltrate and remove cellular debris [6]. Finally, material production/procurement, purification and processing should be easy and scalable [7, 8].

Biological scaffolds composed of ECM have been shown to facilitate the constructive remodelling of many different tissues in both preclinical animal studies and human clinical applications [9]. Specifically, decellularised tissue reproduces more accurately the structure of the ECM. However, its composition strongly depends on the specific origin as well as on the isolation, decellularisation and purification procedures [3, 10]. Bioprinting would overcome this obstacle, providing reliable biomaterials in every batch.

The methodologies described in this chapter can be considered to be the fundamentals for regenerative medicine and/or medical device purposes, but nevertheless the scope of this chapter is not the development of such procedures. Instead, the following sections aim to provide an overview and a description of the state of the art of the possibilities for the preparation and clinical application of natural materials in the context of tissue establishments' activities.

31.2. Decellularisation

31.2.1. Opportunities

Decellularised tissue is used with the aim of developing biological substitutes to restore, replace or regenerate damaged tissues [11-13]. A variety of tissues could be used to produce decellularised ECMs, such as heart valves [14-17], blood vessels [18-20], skin [21], nerves [22, 23], skeletal muscle [24], tendons

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15270 [25] or ligaments [26]. These ECMs may be used directly for tissue implantation or can be modified,
15271 depending on the desired application. The resulting scaffolds have several advantages for the effective
15272 treatment and restoration of unhealthy, missing or damaged tissue. In addition, the absence of constituent
15273 donor cells can help to create a micro-environment more conducive to recipient recellularisation and re-
15274 vascularisation *in vivo*. In most tissues, except tissues with an immune-privileged niche, such as cartilage
15275 or eye, the removal of components represents a significant improvement of graft compatibility and
15276 enhances transplantation outcomes through the reduction of immunogenicity [27].
15277 Additional manipulations of ECM are outside the scope of this Guide, but they include, for
15278 example:
15279 • scaffold recellularisation, in cases where it is necessary to combine manipulation of ECM with
15280 cellular therapy,
15281 • use in conjunction with a chemical molecule (drug, growth factor or protein) in cases where
15282 localised delivery is a must,
15283 • chemical treatment, in cases where it is needed to change the biomechanical properties of the
15284 tissue.

15285 Whenever the manipulation of ECM leads to a substantial manipulation, or when cells and tissues
15286 are not intended to be used for the same essential function or functions in the recipient as in the donor
15287 [28], such use is not considered to be within the scope of European Tissue and Cells Directives, but
15288 classified as advanced therapy medicinal products (ATMPs) instead. Legal requirements for the
15289 development of ATMPs are not addressed in this Guide. Information on regulations and requirements
15290 applicable to development of cell technologies can be found in Chapter 28.
15291 The resulting scaffold from decellularisation is composed of ECM molecules – secreted by the
15292 resident cells of each tissue – which provide biological properties and are organised into a three-
15293 dimensional (3D) arrangement that confers mechanical and structural properties. The ECM is an
15294 interconnected network composed of proteins, lipids, proteoglycans and, in some cases, inorganic salts
15295 such as occur in the bone matrix [29]. The recellularisation of the scaffold can be produced by cells
15296 surrounding the tissue or by cells that have been seeded previously *in vitro.* These cells will be
15297 responsible for generating the new ECM, promoting tissue regeneration [30].
15298 Decellularisation procedures usually represent a greater complexity than the traditional processes
15299 developed in tissue establishments. Therefore, before implementing decellularisation techniques, special
15300 attention should be given to activities planning because decellularisation procedures may have a
15301 significant impact on daily activities, since they often require the use of classified areas for a period of
15302 several days.
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15305 **Table 31.1. Biomaterials used now or under study to be used as scaffolds for different applications [31]**

Tissue	*Biological*
Bone	autologous grafts, alginate, chitosan, collagen, fibrin, gelatine
Articular cartilage	alginate, agarose, chitosan, collagen, fibrin, gelatine, hyaluronan
Heart	alginate, agarose, chitosan, collagen, elastin, fibrin, gelatine, hyaluronan, laminin, platelet gel, silk, starch, vitronectin
Pancreas	alginate, agarose, chitosan, collagen, laminin
Vasculature	alginate, agarose, chitosan, collagen, elastin, fibrin, gelatine, hyaluronan
Skin	collagen, glycosaminoglycans, hyaluronan
Nerve	collagen, fibrin
Ocular	collagen, fibrin, amniotic membrane

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15307
31.2.2. Challenges

15308 The structures of any scaffold should incorporate the appropriate biophysical, biomechanical and
15309 biochemical cues that guide cell proliferation, differentiation, maintenance and function [8]. The
15310 complexity lies in the fact that each product should focus on the specific clinical application, and
15311 therefore different scaffolds with different properties need to be delineated [32, 33].
15312 Regarding physical signalling, the scaffold internal structure – e.g. size and interconnectivity of
15313 pores – determines not only the transport of nutrients, metabolites and regulatory molecules [32, 34], but
15314 also the accommodation of the cells and their organisation into functional tissues. For instance, it has
15315 been largely demonstrated that extremely large pores could avoid vascularisation (endothelial cells are
15316 not capable of bridging pores larger than their diameter), but pores smaller than 100 µm can influence
15317 diffusion. Therefore, from the point of view of the cells, both the micro-environment and the macro-
15318 environment should be taken into account. From the chemical perspective, the scaffold has an important
15319 role in cellular attachment and differentiation. Specifically, it has been reported that properties such as
15320 wettability, charge, chemistry, roughness and stiffness play an essential role in determining whether cells
15321 are able to adhere. All these properties must be taken into account in the biomaterial selection or can be
15322 achieved through specific modifications.

15323
31.2.2.1. Evaluation of the decellularisation set-up

15324 During the set-up of a decellularisation process there are many different parameters to take into
15325 account and analyse, with the aim of assuring the maintenance of the biological and mechanical
15326 characteristics of the tissue. These parameters are analysed during the set-up of the protocols and their
15327 validation, but not all of them will be transferred and implemented as quality controls of the released
15328 tissues. Moreover, the set of parameters to be analysed should be defined for each decellularised tissue,
15329 depending on the final specifications of the product.
15330 As the aim of decellularisation is to preserve the native properties of the tissue while eliminating
15331 the cell remnants that could elicit a host immune response, it is necessary to evaluate such parameters
15332 as the removal of cells, the elimination of genetic material, quantification of ECM protein content and
15333 the mechanical properties in order to assess the quality of the decellularisation protocol [35, 36].
15334 Furthermore, it is necessary to ensure that toxicity resulting from the implantation of SoHO scaffolds is
15335 not a risk for the host.

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Although complete decellularisation (with the elimination of 100% of cell material) may be impossible to achieve, it is convenient to evaluate the effectiveness of the decellularisation protocols with some minimal criteria, such as cell removal and the accepted residual DNA [35-39].

31.2.2.2. Microbiological assessment of natural scaffolds

Sterilisation techniques may be applied to provide a higher level of safety, since scaffolds contain no living cells; however, classic sterilisation methods, such as ethylene oxide exposure [40-42], gamma irradiation [43-47] and electron-beam irradiation [48-50], are known to alter ECM structure and mechanical properties, which are critical for ECM functionality [51, 52]. Additionally, ethylene oxide treatment can cause undesirable host immune responses that impair proper functioning of the biological scaffold after implantation. Therefore, sterilisation by ethylene oxide is only acceptable when there is no other suitable alternative (*Ph. Eur.* 5.1.1).

ECM can be sterilised by simple treatments with acids or solvents, but such methods may not provide sufficient penetration within the tissue depending on thickness and matrix components. Also, as explained above, these methods are aggressive and (depending on time and temperature of the procedure) may damage key ECM components. There are significant advantages to combining the decellularisation and sterilisation processes to ensure a clinically safe ECM for the recipient without affecting its ECM structure. At the moment, paracetic acid has been shown to be the best solution to minimise bacterial, fungal and spore contamination [53]. Tributyl phosphate organic solvent has viricidal properties [54].

Supercritical carbon dioxide is under investigation as a method for the sterilisation of natural ECM [55, 56]. This agent reduces the bacterial and viral loads, with minor changes in mechanical properties relative to other sterilisation methods.

Absence of bacteria and other microbial contaminants, which is achieved by a correct process of sterilisation, is not the only condition required to avoid an undesired patient response caused by pathogens. Endotoxins in the ECM may induce strong biological responses in the patient, fever being the main manifestation (see Chapter 10 for detailed endotoxin testing guidance). Although these bacterial endotoxins are among the most frequent pyrogens, other micro-organisms – such as mycobacteria, fungi and viruses – also release pyrogens. Depyrogenation agents [41, 42] to eliminate endotoxins, and any intact viral and bacterial DNA that may be present, should be applied when indicated. Because pyrogens are often difficult to remove from ECM, inactivation or destruction may be preferable. Acid–base hydrolysis, oxidation, heating or sodium hydroxide are frequently used to this end [41, 42, 57]. However, careful evaluation should be made to avoid affecting the mechanical or biological properties of the ECM.

31.2.2.3. Quality control of decellularised tissues

The quality control and acceptance criteria for tissues that will be decellularised should be adapted, taking into account the purposes of the decellularisation. For instance, tissues with low levels of microbial contamination may be considered acceptable for decellularisation, as long as the process can be validated to remove such contamination. When the donor meets all the criteria, but any human pathogen is detected in the tissue sample at any stage, further processing must be undertaken, even if the tissue is sterilised throughout the process. These measures should be based on an validated risk-assessment algorithm.

The quality control of ECM scaffold after the decellularisation process should consider the following:

a. effective removal of cells and cellular components (see Chapter 8);
b. effective removal of microbial contamination (see Chapter 10) and any potentially toxic microbial products (e.g. endotoxins);
c. effective removal of undesirable and potentially toxic reagents;
d. maintenance of desired ECM structural characteristics.

15383 # 31.3 Bioprinting

15384 ## 31.3.1. Opportunities

15385 The ability to procure an effective custom-made graft, with specific shape, size, porosity and
15386 mechanical properties, is of great interest for personalised medicine to treat specific patient pathologies.
15387 Ideally, the composition of this *de novo* built graft should accomplish certain specific needs, such as the
15388 induction of new tissue formation and regeneration, without activating the immunological response [58].
15389 Several studies show that decellularised ECM from different tissues can promote regeneration in
15390 damaged areas, such as demineralised bone [59], amniotic membrane [60], nerve [61] and skin [62, 63].
15391 In recent years, due to the biological composition of ECM, the use of decellularised tissues as raw
15392 material has been considered for the generation of an ECM-based biomaterials pool to develop bionics
15393 in 3D bioprinting. The main hypothesis is that, if decellularised ECM promotes tissue regeneration, so
15394 the ECM pool from digested tissue could be used as the raw materials in a 3D bioprinting technique to
15395 develop *de novo* personalised grafts.
15396 Bioprinting is an emerging methodology that allows the generation of 3D structures by controlled
15397 disposition of biological materials and functional elements as biochemicals or living cells. The technique
15398 itself arranges these components layer-by-layer with a particular spatial placement of functional
15399 components [64]. The final aim of the technique is to mimic, to the maximum extent, the natural
15400 environment of live tissues at both structural and cellular levels. Importantly, the combination of natural
15401 biomaterials (that possess the appropriate biochemical and biomechanical signals) with bioprinting
15402 (which can design complex structures with specific shape and size at macroscopic and microscopic
15403 levels) opens the door to obtaining a new set of tissues with defined and specific characteristics.
15404 Nowadays, 3D bioprinting is being explored in the regenerative medicine field as a way to tackle
15405 the need for tissues and organs suitable for transplantation. Importantly, recent advances have enabled
15406 3D bioprinting of biocompatible materials, cells and supporting components to generate and transplant
15407 complex 3D functional living tissues. For example multilayered skin, bone, vascular grafts, tracheal
15408 splints, heart tissue and cartilaginous structures have been implanted [64]. Moreover, this technique
15409 offers the opportunity to combine different natural materials to obtain hybrid scaffolds, in order to
15410 enhance bioactivity in the implantation site.
15411 Decellularised ECM has been used for bioprinting [54-67], as well as several ECM components
15412 such as collagens [68], fibrin [69] or gelatine [70]. The advantage of using natural polymers is that they
15413 fully satisfy the biochemical requirements of the tissue in terms of composition and biochemical
15414 signalling. Nowadays, the natural polymers used are mainly collagen and gelatin obtained from tendons
15415 and ligaments from tissue banks. This approach allows clinicians and patients to benefit from the
15416 biological properties of human tissues that have been increasingly used in recent decades, and gives the
15417 opportunity to tissue establishments to integrate the new 3D bioprinting technologies for more
15418 personalised grafts.

15419 ## 31.3.2. Challenges

15420 3D bioprinting involves different levels of complexity, such as the choice of bio or synthetic
15421 materials, cell types, or growth and differentiation factors, and technical challenges related to the
15422 sensitivities of living cells and the rheology of the raw materials for the construction of the tissues.
15423 Dealing with these complexities requires a combination of different fields of expertise, from engineering
15424 and biomaterials science to cell biology, physics and medicine [64].
15425 The process of bioprinting is composed typically of various steps: imaging of the native tissue or
15426 organ, design approach, material and cell selection, bioprinting itself and application [64]. Clinical trials
15427 have been made with bioprinting technology for the regeneration of tracheal [71] and craniofacial defects
15428 [72].

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15429 But it must be repeated that these approaches have not yet been deeply studied for human
15430 application. Therefore, a lot of effort is still needed in this field to improve the procurement of tissues
15431 and organs useful for transplant.

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Related document:

Appendix 33. Decellularisation.

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Chapter 32. Somatic cells in clinical use

32.1. Introduction

Advances in medical research and the developing field for clinical applications using somatic cells for autologous or allogeneic therapies hold great promise for patients with a wide range of serious diseases. These therapies rely on a supply of cells of appropriate safety and quality. This chapter provides guidance for tissue establishments on quality and safety aspects, not only of donation, procurement and testing of the starting material but also in the further processing, storage and release of the cells. The general quality and safety demands in Chapters 1 to 16 (Part A) apply, but some specific considerations for these cells are also relevant. The chapter also aims to provide an overview of some of the cellular therapies used in the clinic but still under further development. The special considerations for donor selection, procurement, certain quality criteria, biovigilance, storage and administration are described in these sections, and an overview of the different steps is provided in Tables 32.1 and 32.2.

In the EU, some of the cell preparations described in this chapter fall under the definition of an advanced therapy medicinal product (ATMP). Such cell preparations are governed by specific requirements and procedures, including prior authorisation by the competent authority, i.e. the medicinal product agency. When ATMP preparation takes place in the EU, or where products meeting the ATMP classification are intended to be used in the EU, their processing, quality control, storage, packaging, distribution, traceability and use must be done in accordance with medicinal product legislation, specifically EC Regulation 1394/2007. In these cases, donation, procurement and testing of such cells must comply with the requirements in Directive 2004/23/EC. For all other requirements, full EU Good Manufacturing Practice (GMP) must be applied.

Any operator intending to process, store, distribute or use cells which might be considered to be ATMPs should seek advice from their national competent authority. In case of doubt whether a specific cell-processing activity is regulated under the ATMP regulation, a recommendation from the Committee for Advanced Therapies (CAT) can be requested. For further details of EU legislation for ATMPs (including their manufacture, storage, distribution, labelling, advertising, traceability and use), see Chapter 30.

The following chapters of this Guide all apply to these cells and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, risk management and validation (Chapter 2);
c. Recruitment of potential donors, identification and consent (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Premises (Chapter 7);
g. Principles of microbiological testing (Chapter 10);
h. Distribution and import/export (Chapter 11);
i. Organisations responsible for human application (Chapter 12);
j. Computerised systems (Chapter 13);
k. Coding, packaging and labelling (Chapter 14);
l. Traceability (Chapter 15);
m. Biovigilance (Chapter 16);

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n. Developing cell technologies (Chapter 30);
o. Preparation of natural scaffolds (Chapter 31).

In an attempt to offer to the reader a more comprehensive approach to the different cell therapies included in this chapter, they have been split into two parts:

a. somatic cells employed to restore or produce immunological functions in the patients:
   i. antigen-specific T-cells,
   ii. natural killer cells,
   iii. dendritic cells,
   iv. mesenchymal stem cells,

   and
b. somatic cells employed to restore organ- or tissue-specific functions:
   i. mesenchymal stem cells,
   ii. chondrocytes,
   iii. keratinocytes,
   iv. limbal stem cells (ocular surface),
   v. stromal vascular fraction from adipose tissue.

Sections 32.2 to 32.5 examine group a: somatic cells employed to restore or produce immunological functions in patients.

32.2. Antigen-specific T-cells

32.2.1 General introduction

Autologous or allogeneic antigen-specific T-cells directed to pathogens or tumour cells may be obtained either through enrichment by cell culture in the presence of a specific antigen or by direct selection. Technology is available for the capture and isolation of cells based on the affinity of cell-surface receptors for specific proteins or peptides immobilised on a suitable insoluble matrix. This technology can be used to isolate donor T-cells from peripheral circulation with specific affinity for pathogenic or other antigens that can then be transplanted to elicit a beneficial immune response in the recipient (adoptive immuno-therapy).

For example, viral infection in immuno-compromised patients after haematopoietic stem cell or solid organ transplantation is a frequent cause of morbidity and mortality. It has been possible to reconstitute the anti-viral immunity of the recipient against specific viruses – e.g. *Cytomegalovirus*, Epstein–Barr virus and *Adenovirus* – through isolation and adoptive transfer of autologous (solid organ) or donor-derived virus-specific T-cells. Also, pre-established virus-specific T-lymphocytes from allogeneic HLA-typed third-party donors may be used to treat virus-induced disease after HLA-mapping and selection of a suitable HLA-match [1]. If such banked virus-specific T-cells are to be used, an assessment for the risk of graft *versus* host disease (GvHD) or graft rejection, and an assessment for efficacy, based on the degree of HLA-(mis)match, must be considered by a qualified specialist in immunology and allogeneic stem cell transplantation.

T-cells can also be modified, using gene-transfer technology (see Chapter 30), to express high-affinity natural T-cell receptors or antibody-like receptors to selected antigens. The latter are synthetic proteins normally consisting of single-chain variable fragments (scFv) of an antigen-specific antibody fused with other proteins to ensure that it is displayed on the surface of the T-cell with appropriate transmembrane activity and effector properties in response to the desired target. Because these synthetic receptors consist of a fusion of different proteins, they are known as chimeric receptors, and T-cells modified in this way are called chimeric antigen receptor-T (CAR-T) cells [2]. Because of the need for appropriate expression and processing of the chimeric proteins in the host cells, integrating retroviral or lentiviral gene vectors (see Chapter 30) are commonly used for gene transfer to create CAR-T-cells,

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15668 although physical methods based on electroporation have also been successfully employed. This
15669 approach is used for cellular immuno-therapy of cancer when sufficient naturally occurring antigen-
15670 specific T-cells cannot be isolated from an individual, or to overcome the consequences of immune
15671 tolerance on endogenous tumour-specific T-cell repertoire.
15672 Clinical trials with such engineered CAR-T-cells represent a promising development of specific
15673 anti-tumour responses targeting diverse antigens in blood cancers as well as in solid tumours; for a brief
15674 overview, see Table 32.1 [2, 3]. Until 2018, two CAR-T-cell therapies had been approved by the Food
15675 and Drug Administration and the European Medicines Agency, one therapy for the treatment of children
15676 with acute lymphoblastic leukemia and the other for adults with advanced lymphomas. Now there are
15677 also pre-clinical advances in CAR design that argue favourably for the advancement of CAR therapy to
15678 tackle other haematological malignancies as well as solid tumours. [4].

15679 **32.2.2 Donor selection**

15680 Donors should be tested for transmissible diseases in accordance with Chapter 5; in addition, the
15681 presence of circulating antibodies against the specific target antigen should be determined before assays
15682 for specific T-cells are initiated.
15683 The patient's own cells are normally used for CAR-T-cell therapy, but stem-cell donors or third-
15684 party donors are also employed. As well as the transmissible disease testing just mentioned,
15685 consideration should be given both to the possible presence of a wild-type virus of the same type as the
15686 basis of the gene vector employed and to the likelihood of the formation of a replication-competent
15687 virus.

15688 **32.2.3 Procurement**

15689 Mostly, antigen-specific therapeutic T-cells are selected from an apheresis product following the
15690 specifications described previously (see Chapter 22). In the case of selection by culture, a sample of
15691 heparinised venous blood (50-60 mL) from a stem cell donor may be sufficient to obtain T-lymphocytes
15692 specific for viruses that elicit high-frequency memory T-cells (Epstein–Barr virus, *Cytomegalovirus*).
15693 For CAR-T-cells, a sample of heparinised venous may be enough, since the CAR-T-cells are then
15694 expanded *in vitro* and *in vivo*. However, the mononuclear cell fraction isolated through an apheresis
15695 process is normally used as starting material to ensure a higher dose of T-cells for cell culture.

15696 **32.2.4 Quality control**

15697 The specific requirements for release include potency assays to determine antigen-specificity
15698 according to pre-established criteria (i.e. IFN-γ production that can be quantified using ELISpot assay
15699 or flow cytometry; or lytic activity that can be quantified by chromium-release assay or by flow
15700 cytometry) and identity by phenotype using flow cytometry. Evaluation of contaminants by flow
15701 cytometry should also be included. Expected viability after thawing should also be established if the
15702 specific T-cells are stored frozen for repeated *in vivo* transfer. Where T-cells expanded in cell culture are
15703 used for cancer treatment, dosing may have to be synchronised with chemotherapy and the cells may
15704 need to be available freshly prepared. This may require a planned two-phase release (see Chapter 27).

15705 **32.2.5 Storage and distribution**

15706 Cultured and released cells can be cryopreserved < −140 °C in liquid or vapour-phase nitrogen, or
15707 deep-frozen with liquid nitrogen back-up. In case of expected storage for prolonged periods, stability
15708 testing may also be considered.
15709 Precautions should be taken to prevent cross-contamination of infectious agents if stored in the
15710 liquid phase of nitrogen, including the use of double containers.

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15711 #### 32.2.6 Traceability

15712 Records, with all information from procurement to *in vivo* administration, should be kept by the
15713 tissue establishment.

15714 #### 32.2.7 Biovigilance/pharmacovigilance

15715 Whenever adverse events occur during the processing – or adverse reactions during application –
15716 of the T-cells, this should be documented and reported (see Chapter 16). As an example, T-cells that do
15717 not fulfil criteria for specificity as measured by *in vitro* methods can still be used as they may fulfil the
15718 biological effect measured *in vivo*.

15719 The most common adverse effects and complications of this cell therapy are during cell
15720 application. Some of the adverse effects and complications are very serious and life-threatening, and
15721 require specific attention and urgent reporting, e.g. cytokine release syndrome, severe neurological
15722 toxicity and insertional oncogenesis [5].

15723 ### 32.3 Natural killer cells

15724 #### 32.3.1 Introduction

15725 Natural killer (NK) cells were described, first in mice, and later in humans as non-B, non-T
15726 lymphoid cells with a non-major histocompatibility complex (MHC)-restricted cytotoxic activity against
15727 transformed or virally infected cells. The 'missing-self' theory by Kärre *et al.* [6] and the identification
15728 of killer Ig-like receptors (KIR) acting as inhibitory or activating signals have contributed to the
15729 understanding and better design of clinical trials. NK cells are bone-marrow-derived from $CD34^+$
15730 progenitors, and migrate upon differentiation to lymphoid organs and peripheral blood. Their
15731 development and homeostasis are dependent on IL-15, and they express the adhesion molecule CD56
15732 but lack the T-cell receptor and CD3. NK cells can be divided into at least two subpopulations according
15733 to their surface density of CD56 expression:

15734 a. $CD56^{bright}$ in a resting stage are considered to be regulatory NK cells that produce high levels of
15735 cytokines and are more proliferative, but poor mediators of NK cell cytotoxicity;

15736 b. $CD56^{dim}$ in a resting stage are potent cytotoxic cells mediating NK cytotoxicity as well as
15737 antibody-dependent cytotoxicity through CD16 (FcγRIII).

15738 NK cells can be activated by several cytokines and they produce a wide variety of cytokines and
15739 chemokines: granulocyte-colony stimulating factor (G-CSF), tumour necrosis factor (TNF)-α and TNF-
15740 β, IFN-γ, tumour growth factor (TGF)-β, macrophage inflammatory protein 1-beta, and regulated on
15741 activation, normal T-cell expressed and secreted (RANTES). It is still not clear whether the *in vivo* effect
15742 of NK cells is a result of direct killing or indirectly through cytokine production, engaging other parts
15743 of the immune system. NK cell-based immuno-therapies against malignancies involve using either the
15744 autologous NK cells *in vivo*, by cytokine stimulation, or by adoptive transfer of autologous or allogeneic
15745 NK cells. There are many different protocols and clinical studies using NK cells against malignancies,
15746 as reviewed by Cheng *et al.* [7], but not covered in this chapter.

15747 In addition to T-cell-mediated immuno-therapy, the unique biology of NK cells makes them a
15748 valid tool for immuno-therapy. In contrast to T-cells, CAR-modified NK cells show less severe side-
15749 effects, such as GvHD, because donor NK cells usually do not attack non-haematopoietic tissues such
15750 as liver, kidney, muscle and lung. A number of clinical trials have shown that NK cell infusion has less
15751 severe GvHD than does T-cell infusion.

15752 Currently, CAR-modified NK cell lines are used as effector cells for various cancer treatments
15753 (acute lymphoblastic leukemias, glioblastoma, neuroblastoma, breast and prostate cancers, multiple
15754 myeloma) and also as immuno-therapy for serious infectious diseases such as HIV [8].

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15755 **32.3.2 Donor selection**
15756 Depending on the clinical protocol, autologous NK cells can be used, either by activation of the
15757 presumed NK cells *in vivo*, or by *ex vivo* selection and/or activation.
15758 Increasing knowledge of MHC recognition (classical or non-classical) and interaction, and of the
15759 haplotypes of KIR, suggests that donor selection for protocols with adoptive transfer of allogeneic NK
15760 cells could include MHC typing for HLA-C, HLA-E, and possibly also HLA-F and HLA-G, as well as
15761 KIR. The aim would be to select a donor whose ligands for the inhibitory signals were absent in the
15762 recipient. For adoptive transfer of *ex vivo*-activated NK cells with additional *in vivo* activation, special
15763 care should be taken to reduce the possible toxic effects of cytokines, depending on the underlying
15764 disease and the dose of the cytokine(s).

15765 **32.3.3 Procurement**
15766 NK cells can be procured from peripheral blood, by apheresis carried out by experienced
15767 personnel, as described in Chapter 22 (see §22.3.2.2).
15768 Smaller amounts of NK cells to be expanded *in vitro* are obtained from 30-50 mL anti-coagulated
15769 venous blood. *In vitro* expanded NK cells can also be obtained from $CD34^+$ stem cells from cord blood
15770 after *in vitro* differentiation and maturation with defined cytokines.

15771 **32.3.4 Quality controls/release criteria**
15772 In addition to microbiological testing as described in Chapter 9, release criteria should include
15773 the defined level of phenotypic NK cells as established by flow cytometry, and possibly also a functional
15774 assay (cytokine production or cytotoxic assay).

15775 **32.3.5 Packaging and distribution**
15776 The processed and released NK cells are aspirated into a syringe for direct administration to the
15777 patient, with labelling containing the recipient identification and, if applicable, the dose of NK cells.
15778 Attached documentation should contain additional information to prevent errors if other patients or
15779 clinical trials are ongoing in the clinic (see Chapters 11 and 14).

15780 **32.3.6 Traceability**
15781 Records, with all information from procurement to *in vivo* administration, should be kept by the
15782 tissue establishment.

15783 **32.3.7 Biovigilance/pharmacovigilance**
15784 Adverse events during the procurement or processing that should be documented could involve,
15785 for example, less NK cell recovery than expected after a cell-separation step or a lack of functional
15786 activity measured *in vitro* as a quality control. Adverse reactions that should be documented could
15787 involve unexpected side-effects related to the administration of NK cells or to the additional activating
15788 cytokines, all of which should be documented.

15789 ## 32.4 Dendritic cells

15790 **32.4.1 General introduction**
15791 Tumour vaccines based on dendritic cells (DC) are a new form of immuno-therapy which is being
15792 tested in a large number of trials internationally [9]. DC have the capacity to activate tumour-specific T-
15793 cells to attack and eliminate the patient's tumour. There are several subtypes of DC vaccines, but most
15794 are derived from monocytes that are cultured in a cytokine mixture composed of GM-CSF and IL-4 and
15795 then induced into mature DC by various maturation factors. See Table 32.1 for an overview.

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15796 ### 32.4.2 Donor selection
15797 The majority of DC vaccines are autologous and derived from monocytes purified from the blood
15798 circulation of the patients. As described in Chapter 5, donor testing includes assays for transmissible
15799 diseases; and, although their presence is not an exclusion criterion, it should be documented and care
15800 should be taken to avoid cross-contamination of other cells or personnel. Medical evaluation of the
15801 patient should take into account the burden of collecting large amount of leukocytes using apheresis.
15802 Depending on the method used to obtain monocytes from the collected leukocytes, a predetermined
15803 level of circulating monocytes may be relevant.

15804 ### 32.4.3 Procurement
15805 As a starting material, apheresis-derived leukocytes are normally used. Apheresis should be
15806 performed by professionals specialised in apheresis, with the precautions mentioned in this chapter for
15807 DLI collection (Chapter 22).
15808 The blood volume processed, in order to obtain a sufficient number of monocytes for further
15809 processing, depends on the patient's peripheral blood counts and should be calculated to avoid
15810 unnecessary apheresis time with the increasing risks of serious adverse reactions.

15811 ### 32.4.4 Quality controls/release criteria
15812 Prior to freezing, the mature DC are tested by flow cytometry for their expression of a number of
15813 cell-surface markers which are characteristic for mature DC. These include markers such as low CD14
15814 expression and high expression of CD80, CD83, CD86, CD1a, HLA-DR, DC-SIGN, ILT-3 and CCR-7.
15815 Secreted markers, e.g. IL-12, can be tested by ELISA assays.

15816 ### 32.4.5 Packaging and distribution
15817 Matured and aliquoted DC are frozen with cryoprotectant in cryo-tubes and stored at −140 °C in
15818 a freezer or in liquid nitrogen in a cell bank. Frozen cells are distributed on dry ice or in liquid nitrogen.

15819 ### 32.4.6 Traceability
15820 Records for the complete process, from donor selection to clinical use, should be kept by the
15821 responsible tissue establishment.

15822 ### 32.4.7 Biovigilance/pharmacovigilance
15823 Any adverse event during procurement of leukocytes, cell separation, culturing, cryopreservation
15824 or distribution, or any adverse reactions during administration, should be documented as described in
15825 Chapter 16. An example of such an adverse event might be lower numbers of monocytes than expected
15826 when collected by apheresis or during the cell-separation procedures. The route of administration of DC
15827 may involve adverse reactions that should be recorded and if possible avoided, or at least managed
15828 according to a risk–benefit assessment.

15829 ## 32.5 Mesenchymal stem cells

15830 ### 32.5.1 General introduction
15831 Mesenchymal stem cells (MSC), also referred to as mesenchymal stromal cells [10], are
15832 multipotent stem cells with immuno-regulatory and regenerative properties. They can differentiate into
15833 a variety of cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells) and adipocytes
15834 (fat cells). This phenomenon has been documented in specific cells and tissues in living animals and
15835 their counterparts growing in tissue culture.
15836 While the terms MSC and bone-marrow stromal cells (BMSC) have been used interchangeably,
15837 neither term is sufficiently descriptive, as discussed below.

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15838 Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that
15839 differentiates into haematopoietic and connective tissue, whereas MSC do not differentiate into
15840 haematopoietic cells.
15841 Stromal cells are connective tissue cells that form the supportive structure in which the functional
15842 cells of the tissue reside. While this is an accurate description for one function of MSC, the term fails to
15843 convey the (relatively recently discovered) roles of MSC in the repair of tissue.
15844 The term MSC, used by many labs today, can encompass multipotent cells derived from other
15845 non-bone-marrow tissues, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp
15846 of deciduous baby teeth; see also Tables 32.1 and 32.2.
15847 The International Society for Cellular Therapy encourages the scientific community in all written
15848 and oral communications to adopt this uniform nomenclature (MSC) when cells meet specified stem
15849 cell criteria. It defines the specific MSC criteria thus:

15850 The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes
15851 minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard
15852 culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34,
15853 CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts,
15854 adipocytes and chondroblasts in vitro. [10]

15855 These criteria will probably change as new knowledge unfolds. MSC are characterised
15856 morphologically by a small cell body with a few cell processes that are long and thin. The cell body
15857 contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed
15858 chromatin particles, giving the nucleus a clear appearance. The cells, which are long and thin, are widely
15859 dispersed; and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of
15860 the other types of collagen fibrils.
15861 The immuno-regulatory and regenerative properties of MSC make them an attractive tool for the
15862 development of treatments of autoimmunity, inflammation and tissue repair [11, 12]. MSC do not induce
15863 alloreactivity but generate a local immuno-suppressive micro-environment by secreting cytokines.
15864 However, MSC interfere with dendritic cell activation, and they suppress lymphocyte activation and T-
15865 cell function *in vitro*. They have been shown to reverse inflammation in several experimental animal
15866 models, and clinical studies indicate that MSC are immuno-suppressive also in humans as they reverse
15867 steroid-refractory GvHD and other inflammatory conditions [12, 13]. MSC are a heterogeneous
15868 population of cells, with functions depending on both source and *in vitro* culturing conditions. MSC are
15869 also investigated for tissue-engineering purposes, mainly for osteo-articular diseases: bone and cartilage
15870 regeneration. The mechanisms behind their tissue-regenerating ability and their immuno-modulating
15871 capacity, and the extent to which the two processes interact, require further elucidation. In view of the
15872 increasing interest in using MSC for human application, the safety and quality aspects to bear in mind
15873 are mentioned in this section.

15874 **32.5.2 Donor selection**

15875 Under resting conditions, MSC express HLA class I but not class II alloantigens. When cultured
15876 *in vitro* with allogeneic lymphocytes, MSC do not stimulate immune responses. Based on these findings,
15877 it has been assumed that MSC can be transfused across HLA barriers; and therefore cells from HLA-
15878 identical siblings, HLA-haplo-identical relatives or third-party HLA-mismatched healthy volunteer
15879 donors have been used in clinical protocols. However, MSC that are to be used for their regenerative
15880 capacity should preferably be autologous.
15881 Donors should be evaluated for their own safety, and for the safety of the recipient, according to
15882 the criteria described in Chapter 4.

15883 ### 32.5.3 Procurement
15884 MSC can be isolated from haematopoietic tissues, such as bone marrow, peripheral blood and
15885 umbilical cord blood, but also from parenchymal non-haematopoietic tissues, such as muscle, fat or
15886 liver. The youngest, most primitive MSC can be obtained from the umbilical cord tissue, namely
15887 Wharton's jelly. Umbilical cord-derived MSC have more primitive properties than other, adult MSC
15888 obtained later in life, which might make them a useful source of MSC for clinical applications.
15889 The two main sources of MSC presently used for human application are bone marrow- and
15890 adipose-derived. The latter is one of the richest sources of MSC: there are about 500 times more stem
15891 cells in 1 gram of adipose tissue than in 1 gram of aspirated bone marrow.

15892 ### 32.5.4 Quality controls/release criteria
15893 At present, release criteria are mostly phenotypic, and include cultures predominately expressing
15894 CD73, CD90 and CD105, although the relevance of these remains to be clarified. Further release criteria,
15895 apart from sterility (absence of bacteria, mycoplasma and fungi; see Chapter 10), is lineage-negativity,
15896 including exclusion of haematopoietic contaminating cells.
15897 It is challenging to identify markers that may be predictive for the potency of a specific product
15898 with a specific indication. However, surrogate markers, such as immuno-modulating capacity or ability
15899 to reconstitute specific tissues or to secrete tissue-specific factors, according to the intended use of MSC,
15900 will be needed to address functional properties and consistency of the cells. It will therefore be necessary
15901 to define and validate the release criteria depending on the culture conditions used and the clinical
15902 protocol.

15903 ### 32.5.5 Packaging and distribution
15904 When distributed to the clinic for administration to the patient, the cells should be either
15905 transferred to vials with the pre-decided dose for administration or distributed directly in a pre-labelled
15906 syringe, depending on whether the MSC are processed in the close vicinity of the clinic.
15907 Frozen vials of MSC are often thawed at the bedside by diluting with isotonic saline solution, at
15908 least 4× volumes (to avoid toxicity by the cryoprotectant to the MSC), and administered immediately to
15909 the patient. A small portion of the thawed, diluted MSC can be used to verify cell numbers and viability.

15910 ### 32.5.6 Traceability
15911 Records to ensure traceability from the donation to the recipient should be kept with the tissue
15912 establishment.

15913 ### 32.5.7 Biovigilance/pharmacovigilance
15914 As indicated above, the MSC consist of a heterogeneous population; their phenotype and function
15915 are dependent on source and culture condition. Accordingly, any deviation from the expected endpoints
15916 (according to the predefined criteria) should be considered an adverse event that should be recorded.

15917 Sections 32.6 to 32.10 (below) examine group b: somatic cells employed to restore organ- or
15918 tissue-specific functions.

15919 ## 32.6 Mesenchymal stem cells

15920 ### 32.6.1 General introduction
15921 Mesenchymal stem cells (MSC) have been used to produce immunological functions or to restore
15922 tissue-specific functions. They have been described (above) in section 32.5.

15923 ## 32.7 Chondrocytes

15924 ### 32.7.1 General introduction

15925 Damaged articular cartilage has a limited capacity for self-repair. Cartilage lesions are usually
15926 associated with disability and symptoms such as pain, swelling, locking and malfunction of the joint,
15927 and if these lesions are left untreated it may lead to osteoarthritis. Autologous chondrocyte implantation
15928 (ACI) is a therapy widely used for the treatment of isolated cartilage defects. The original (first-
15929 generation) technique is based on an implantation of a suspension of *in vitro* expanded chondrocytes
15930 into the defect beneath a sealed cover of periostium flap. Since the technique was introduced in 1987 by
15931 Brittberg *et al.* [14], over 35 000 patients have been treated worldwide.
15932 The second-generation ACI technique includes the use of a collagen membrane instead of the
15933 periosteal flap. The use of collagen membrane simplifies the surgical procedure and reduces
15934 complications such as periosteal hypertrophy.
15935 Further technological advances led to the development of the third-generation technique that
15936 involves both *in vitro* expanded chondrocytes and a scaffold (briefly described in Chapter 29). After the
15937 expansion in culture flasks, the cells are seeded onto a membrane or a biodegradable scaffold before
15938 implantation. The scaffold may function as a carrier for the cells or as a structure to build up the 3D
15939 environment for the cells. In most techniques only fibrin glue is used for the fixation of the graft and,
15940 since there is no need for any suturing of the periostium/collagen membrane cover, this implantation can
15941 be done arthroscopically.
15942 For an overview, see Table 32.2.

15943 ### 32.7.2 Donor selection

15944 The patient is examined by an arthroscopic procedure where the location, depth and size of the
15945 defect and the quality of the surrounding cartilage are evaluated. A typical patient is a young patient with
15946 large (> 2 cm²) full-thickness chondral or osteochondral defects surrounded by healthy cartilage. At the
15947 present time ACI is not indicated for patients with severe osteoarthritis, active rheumatoid arthritis or
15948 active autoimmune connective-tissue diseases, or patients with concomitant malignancies [11, 15].

15949 ### 32.7.3 Procurement

15950 The ACI technique includes a two-stage procedure, with an initial harvest of a cartilage biopsy,
15951 which is sent for chondrocyte culture, followed by a second-stage operation that includes the cell
15952 implantation. A full-thickness cartilage biopsy (about 200-400 mg) is harvested from a low-weight-
15953 bearing area of the knee during arthroscopy. The biopsy is transferred to a sterile transport tube with
15954 biopsy medium. The biopsy tube and blood tubes should then be placed in an outer secondary packaging
15955 that ensures the sterility and maintenance of the temperature, and is approved for transport of biological
15956 substances (see also Chapter 14). The biopsy should be kept cold – at about 5-15 °C – during transport to
15957 ensure the quality of the biopsy specimen. Transport should be sent directly to the tissue establishment
15958 for further processing, which should start within 48 hours. For culture conditions with autologous serum
15959 instead of fetal bovine serum, up to 50 mL of autologous blood should accompany the biopsy.

15960 ### 32.7.4 Quality controls/release criteria

15961 Living cells cannot be sterilised, and therefore it is very important to ensure that all handling of
15962 the product is performed under good aseptic conditions and that all material, media and reagents used
15963 are sterile and endotoxin-free (see Chapter 10). The first sterility test for release is done a few days before
15964 the scheduled implantation, at the last media change, and the second test is made during assembly of the
15965 final product. The final result of a sterility test takes normally 10-14 days and the expiry time for the final
15966 product is normally 24-72 hours, so it is common that the cells are already released and implanted before
15967 the final results are available. However, a preliminary result of the sterility test can normally be given

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15968 after 24 hours and it is upon this result that the cells can be released. An indication of the viability of
15969 cells in suspension is obtained using trypan blue. It is more difficult to test the viability of cells growing
15970 in a 3D construct. There is currently no non-destructive assay available, but other release criteria specific
15971 for chondrocytes are used.

15972 *32.7.4.1 Morphology*

15973 The morphology of the cells can be followed easily during culture using an inverted microscope.
15974 The chondrocytes should be typical of cultured chondrocytes in appearance, and the personnel who
15975 perform this subjective judgment must have good experience in this task and should have reference
15976 pictures of cultures for comparison. The cells lose the phenotype of uncultured chondrocytes during
15977 culture and become more fibroblast-like.

15978 *32.7.4.2 Population doubling*

15979 The cells should undergo only a limited and defined number of population doublings, for example
15980 4-8 population doublings. In order to ensure a proper re-differentiation of the cells, the functional
15981 capacity of the cells after maximal expansion has to be shown, and it is important to record the
15982 population doubling during culture.

15983 *32.7.4.3 Number of cells*

15984 The number of cells must be stated on the product. This can be in form of either the actual number
15985 of cells in the vial or syringe or the number of cells per surface area that have been seeded in the scaffold.
15986 Other labelling requirements are as described in Chapter 14.

15987 *32.7.4.4 Purity*

15988 To determine the possible contaminants in the product, such as synoviocytes or other types of
15989 impurity such as bone cells, the purity of the product is assayed. A representative batch of cells can be
15990 validated for presence of mRNA markers for chondrogenic lineage, like sox9, and lack of (or low)
15991 mRNA expression of synoviocyte-specific genes. Since cells are dedifferentiated during culture, more
15992 specific markers of differentiation are not tested.

15993 *32.7.4.5 Chondrogenic potential*

15994 Attempts to predict the chondrogenic potential of the cells and thus the chondrogenic repair
15995 capacity have been made [16], but currently no clinical potency marker exists. Thus, the functional
15996 properties of the cells and an appropriate surrogate marker are still to be defined, and research is needed.

15997 **32.7.5 Packaging and distribution**

15998 The first-generation product (cells in a suspension) is aseptically filled in syringes or vials. The
15999 third-generation product is aseptically packed in a sterile two-container system. The advantage of having
16000 primary packaging that is also sterile on the outside is that it can be taken directly to the operation table
16001 and be handled by sterile personnel. The product should then be placed in an outer secondary packaging
16002 that ensures the sterility and temperature and that should be approved for transport of biological
16003 substances. Implantation is normally done within 48 hours, depending on the expiry time for the product.
16004 For the accompanying information sent to organisations responsible for human applications, see Chapter
16005 12.

16006 **32.7.6 Traceability**

16007 Records with all information, from procurement to implantation, must be kept. Reference samples
16008 may also be archived.

16009 **32.7.7 Biovigilance/pharmacovigilance**

16010 Several adverse events that affect the growth and differentiation of chondrocytes may occur
16011 during the relatively long *in vitro* expansion period. In addition, if for example the degree of

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contaminating cell types unexpectedly exceeds the release criteria, this should be recorded and managed as described in Chapter 16.

32.8 Keratinocytes

32.8.1 General introduction

The current 'gold standard' of burn care [17] is early tangential excision of eschar, i.e. necrotic tissue, and autologous split-thickness skin grafting to the surgical wound areas. The skin has many crucial functions and the main goal with the treatment is to heal the patient's skin as soon as possible and thus restore its functions to make survival from the burn possible for the patient.

Procurement of skin transplants can be repeated from the same donor site after some weeks of healing but, since every procurement includes a small dermal portion, one can usually not procure more than 2 or 3 times from the same site due to the risk of creating a full-thickness skin wound. However, once 20-30 % of the patient's body surface area is burned, the access to healthy skin for transplantation starts to be limited. Therefore, culture of autologous keratinocytes is often the last resort for the most severely burned patients, and a useful tool in treating significant burns.

Culture of human keratinocytes has been in clinical use since the beginning of the 1980s [18]. Cultured keratinocytes, i.e. epithelial cells, can be used both for autologous and allogeneic treatment of patients as described here (see also Table 32.2). Culture of human cells is, according to EC Regulation 1394/2007, an advanced therapy medicinal product and the manufacture of the cells must therefore follow the requirements in the medicinal product regulations. A manufacturing licence from the medicinal product agency is required.

The skin comprises approximately 5 % epidermis and 95 % dermis (even though sometimes part of the subcutaneous fatty tissue is regarded as part of the skin). The dermis is responsible for the skin's strength and pliability. Cultured skin in most applications today is composed only of epidermal cells (keratinocytes), which restore a new epithelium (epidermis) on the patient, thus closing the wound and contributing to the survival of the severely burned patient. Depending on the depth of the burn (i.e. deep dermal or full-thickness burn), the amount of remaining dermis varies greatly. As a result, the quality of the healed skin, after transplantation of cultured epithelial autografts, varies equally greatly (in structure, function and cosmetics), depending on the residual amount of dermis.

Today there are very few therapies to restore the dermis through ordinary cell culture; this is due, *inter alia*, to the morphological appearance of the dermis, with a substantial extracellular matrix in a three-dimensional structure. Extensive research is needed to develop a tissue-engineered skin consisting of both dermis and epidermis that is usable for wound care. Meanwhile, when the options for skin substitutes are limited, biological acellular allogeneic dermis (prepared from donated skin) or synthetic dermal regeneration templates can be used for reconstruction of the dermis. By applying a 3D biological degradable matrix to surgical wounds, the host's cells will migrate and populate the matrix, starting to develop autologous extracellular matrix while degrading the applied matrix, thereby restoring the dermal part of the skin and improving the quality of the reconstructed skin [19] (see also Chapter 19).

Cultured keratinocytes can be used in two ways, either for permanent skin cover in an autologous manner, or contributing to healing in an allogeneic situation through the natural growth-stimulating properties of the cells.

Autologous keratinocytes were until recently cultured and guided into stratified growth, rendering keratinocyte sheets, which can be grafted in the same manner as split-thickness skin grafts [20]. Today a common practice is to culture keratinocytes in multiplicity and mix the cell suspension with tissue glue, to be sprayed onto the wound in a single-cell suspension [21, 22]. Epidermal cell suspensions without culturing, containing keratinocytes, melanocytes and fibroblasts, can also be applied as spray to restore epithelialisation *in vivo* (see §19.11.1).

In the allogeneic use of cultured keratinocytes on full-thickness wounds, the cells make up a temporary skin that will eventually be replaced by the patient's own skin (either split-thickness skin

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16060 grafts or cultured epithelial autografts). With the use of allogeneic keratinocytes on superficial dermal
16061 wounds, the healing time can be shortened and – since the wound is superficial – the possibility of
16062 spontaneous re-epithelialisation is good.

16063 **32.8.2 Donor selection**

16064 For autologous use, donor-site selection and timing are important. To get access to as many adult
16065 progenitor cells as possible, the donor site should preferably be in a hair-bearing area of healthy skin.
16066 The sooner (after trauma) the skin biopsy is taken, the better because the patient (and tissue) will be
16067 contaminated by microbes, which will affect the subsequent cell culture. However, the skin areas
16068 available for donor-site selection are principally determined by the extent and location of the burns.

16069 For allogeneic application, donor selection must include – apart from general donor evaluation
16070 criteria (see Chapters 4 and 5) – the tissue-specific criteria defined in Chapter 19. The transplanted
16071 allogeneic keratinocytes will be a temporary wound coverage, stimulating wound healing, and thus
16072 human leukocyte antigen (HLA) typing or ABO (AB0) blood grouping are not necessary.

16073 **32.8.3 Procurement**

16074 When procuring the skin for culture of keratinocytes, it is essential that the site for the biopsy is
16075 located in an area with healthy skin as remote from the burned skin as possible. The biopsy can be either
16076 full-thickness or split-thickness. A full-thickness skin biopsy is preferred because of the amount of
16077 progenitor-cell-like keratinocytes in the appendages (hair follicles, sweat glands, etc.). The procurement
16078 should yield as many non-differentiated keratinocytes as possible.

16079 The biopsy site should first be cleaned properly with disinfectant ethanol (70 %) with no additives
16080 and, after the site has dried, given a second wash with sterile saline solution (9 mg/mL) before the biopsy
16081 is procured (with e.g. a scalpel). Local anaesthetics can be used *ad lib*. Immediately after procurement,
16082 the biopsy is placed in a medium suitable for transportation to the tissue establishment (basal culture
16083 medium with the addition of 10 % fetal bovine serum – or similar – and antibiotics in normal cell-culture
16084 concentrations). The primary container should be sterile, closed and appropriately labelled. Transport
16085 the biopsy to the culture facility and initiate the cell-isolation process as soon as possible, < 24-48 hours
16086 after surgical removal of the biopsy. Minimizing the time will increase the likelihood of the successful
16087 culture of cells.

16088 It is crucial to keep in mind that handling and culturing the skin biopsy/keratinocytes *in vitro*
16089 opens up a risk of contributing (microbiological) contaminants to the cells, both from the skin itself and
16090 from culture conditions. Those risks can only be avoided by adequate facilities with controlled
16091 environment (see Chapter 9), and skilful, excellent handling techniques by the staff (see Chapter 27).

16092 **32.8.4 Quality controls/release criteria**

16093 The quality controls needed, besides microbiological controls (see Chapter 10), include
16094 continuous surveillance: of keratinocyte morphology, mode of growth and expansion rate. It is important
16095 to have cellular expansion that corresponds to the patient's need for cultured keratinocytes, but it is
16096 equally important not to exceed the cells' capacity to proliferate. The possibility of proliferation within
16097 the keratinocytes is determined by different biological aspects, including the age and healthiness of the
16098 patient (prior to the trauma) and the biopsy site.

16099 **32.8.5 Packaging and distribution**

16100 The packing procedure is determined by the mode of delivery, i.e. sheets or suspension. Sheets
16101 are attached to an inert synthetic carrier membrane and placed one by one in transport liquid consisting
16102 of a basal cell-culture media (e.g. Leibovitz 15). Keratinocytes delivered for spray application are
16103 enzymatically detached from the culture vessel, washed repeatedly in basal cell-culture media and
16104 finally diluted in a minimal amount of basal cell-culture media, just enough to be in a solution. Further

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dilution is performed with the tissue glue to reach the appropriate cell concentration at the surgery room, just before application of the cells. The extent of dilution depends on the number of cells needed to cover the burned area of the patient.

The keratinocytes, either in sheets or in suspension, must be applied as soon as possible (or within a maximum of approximately 6 hours) after preparation.

32.8.6 Traceability

Records to secure traceability from donor to recipient, and all steps in between, are the responsibility of the tissue establishment, as described in Chapter 15.

32.8.7 Biovigilance/pharmacovigilance

As described in Chapter 16, adverse events and adverse reactions must be documented and reported. One can foresee that any event in the laboratory affecting the culture conditions, and thus reducing the amount of cells or the size of cell layer expected on the day of transplantation, should be considered as an adverse event.

32.9 Limbal stem cells (ocular surface)

32.9.1 General introduction

In the normal human eye, the epithelial cells of the cornea and conjunctiva are responsible for the continuing regeneration and homeostasis of the ocular surface. Cells with progenitor characteristics have been identified in the corneoscleral limbus (the transitional zone between the cornea and sclera) and these have the capability to continually repair and replace the epithelial surface of the cornea [23]. These cells are known as limbal stem cells (LSCs). Limbal stem cell deficiency, a term which covers acquired pathological deficiencies such as chemical burns and inherited ones such as aniridia, may lead to ocular surface disease, including persistent epithelial defects with chronic inflammatory conditions, vascularisation and scarring of the cornea and conjunctiva, and corneal conjunctivalisation [24]. These anomalies can be painful and are very difficult to treat because of the significant impairment of the patient's vision which, in most cases, progresses to legal blindness. LSCs have the characteristics of undifferentiated multipotent stem cells and are capable of undergoing proliferation and differentiation into the corneal epithelium. They may also be genetically modulated for therapeutic purposes [25].

Currently, approaches to the treatment of many ocular surface diseases focus mainly on three strategies: transplantation of portions of keratolimbus (Kenyon technique), either from the healthy fellow eye or from a cadaveric donor; *ex vivo* LSC expansion from a limbal explant; *ex vivo* expansion of isolated LSCs and *in vitro* culture. Achieving effective control of the underlying inflammatory process and preventing tissue rejection are the key objectives [24, 25]. In addition to LSCs, corneal endothelial cells [26] derived from the corneoscleral disc are being used for treatment of corneal endothelial disease; see Chapter 17 and Table 32.2. Recently, retinal pigment epithelial cells [27], induced to differentiate from different sources of pluripotent stem cells, have been investigated for the treatment of age-related macular degeneration; see Table 32.2 for an overview.

32.9.2 Donor and tissue selection

Donors should be tested for infectious diseases as described in Chapter 5, although in the case of autologous transplantation a positive test result may not be an exclusion criterion.

The treatment of unilateral LSC deficiency involves *ex vivo* expansion of a tissue explant or isolated LSCs from the unaffected limbal region of a patient's healthy eye. The autologous cell population is isolated and the final aim is to expand the limbal epithelial cells for transplantation into the affected eye. Human amniotic membrane or human fibrin gel are typically used as a scaffold for supporting the *ex vivo* expansion of LSC and used as a carrier for the transplantation of the cells (see

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16149 Chapter 31). Bilateral LSC deficiency, on the other hand, is a devastating pathological condition affecting
16150 both eyes; in this case, autologous limbal tissue or cells cannot be sourced from the same patient since
16151 both eyes are affected. Alternative sources of transplantable tissue include the culture of epithelial cells
16152 lining the autologous oral mucosa. Allogeneic sources of transplantable tissue, from deceased donors,
16153 may be an option for restoring the function of the ocular surface, although procedures with autologous
16154 healthy tissue are always preferable to those using heterologous sources [28, 29].

16155 **32.9.3 Procurement**

16156 Extraction as well as *ex vivo* expansion/*in vitro* cultures must be carried out in strict laboratory
16157 conditions.

16158 The explants (autologous or allogeneic) are obtained by a biopsy of healthy ocular tissue from the
16159 limbal region and maintained in sterile culture medium until processed. The most widely applied
16160 technique, with minimal manipulation of tissue, is the explant culture system, in which the specimen
16161 obtained by biopsy is directly placed in the centre of the amniotic membrane.

16162 Epithelial cells (LSCs) grow out from the biopsied tissue and proliferate to form a multi-layered
16163 epithelial construct.

16164 An alternative approach, with additional manipulation of the tissue, is to obtain single epithelial
16165 cell suspensions by enzymatic isolation (a one- or two-step enzymatic method) from limbal specimens.

16166 **32.9.4 Quality controls and release criteria**

16167 Microbiological tests that simulate normal processing conditions by replacing the cells with
16168 culture media are essential. Three simulations of all the critical stages of the process should be performed
16169 before the implementation process [30].

16170 Conventional microbiological (bacteria and fungi) assays should be performed at baseline, during
16171 the process and in the final product before clinical use. Specific tests for the presence of *Mycoplasma*
16172 spp. are conducted after handling the final product (see Chapter 9). Morphological control of culture
16173 cells and bioengineered products can be achieved with the use of a phase-contrast microscope. Basic
16174 cell-culture techniques can be applied, such as cell viability tests with vital dyes and techniques for cell
16175 counting using an automated cytometer or cell-counting chambers.

16176 In the explant culture system the final product requires less handling, and adequate
16177 microbiological control of the final product (LSCs on amniotic membrane) is needed to obtain approval
16178 for clinical use. Further release criteria are to be established during the pharmaceutical and clinical
16179 development of the cell-based product. In the enzymatic approach, in addition to the controls mentioned,
16180 the cell product may be tested for the presence of holoclones (clonogenic assay), high expression of the
16181 transcription factor deltaNp63alpha or ABCB5 and negative or very low expression of markers both for
16182 differentiated epithelial cells CK3 and CK12 and, where used as a feeder layer for LSC expansion,
16183 markers or PCR-based assays for the presence of 3T3 murine fibroblasts [28, 29].

16184 **32.9.5 Packaging and distribution**

16185 The purpose of packaging and labelling operations is to protect the product and to provide
16186 identification and information for the user. Packaging and labelling are key elements in the quality
16187 management system to ensure that appropriate standards are maintained during a product's life cycle
16188 [30].

16189 The final cell product should be conditioned in a suitable sterile container immersed in
16190 unprocessed complete cell-culture medium and maintained at an appropriate and controlled temperature
16191 until arrival at the transplant centre (see also Chapter 14). A maximum period should be set for delivery
16192 and it is recommended that the product is implanted on the same day as its release for clinical use, unless
16193 longer storage is adequately justified. As described in Chapter 12, the essential information about usage
16194 of the cells should be included in accompanying documentation to the organisation responsible for
16195 human application.

16196 **32.9.6 Traceability**

16197 Special consideration should be given to the reagents that come into contact with tissues and cells,
16198 and which may be left as residues when cells are applied in the recipient. The following links in the
16199 process are particularly important: between the donor and donation at the tissue centre; between donation
16200 and the final cell product at the manufacturing site; and between the final product and the recipient at
16201 the transplant centre. All records and information should be kept and stored at the tissue establishment(s)
16202 or according to national legislation.

16203 **32.9.7 Biovigilance/pharmacovigilance**

16204 As described in Chapter 16, deviations from standard operating procedures, from donation to
16205 clinical application, should be recorded and documented, as well as adverse reactions after application.

16206 ## 32.10 Stromal vascular fraction from adipose tissue

16207 **32.10.1 General introduction**

16208 Adipose tissue is a source of stromal cells similar to those identified in bone marrow. The so-
16209 called stromal vascular fraction (SVF) isolated from fat is a heterogeneous cell population that includes
16210 endothelial cells (10-20 %), haematopoietic lineage cells (25-45 %), stromal cells (15-30 %) and pericytes
16211 (3-5 %), as well as adipose stromal/stem cells (1-10 %). SVF may be used either directly or as a source
16212 material to isolate regenerative cells for treating various clinical conditions including musculoskeletal,
16213 neurological, immunological, cardio-pulmonary and immunological disorders, as well as soft tissue
16214 defects [30-32].

16215 **32.10.2 Donor selection**

16216 Commonly, SVF cells are used autologously. Donor testing includes assays for transmissible
16217 diseases as described in Chapter 5; although their presence is not an exclusion criterion, it should be
16218 documented and special actions should be taken to avoid cross-contamination to other cells and to ensure
16219 the safety of personnel.

16220 **32.10.3 Procurement**

16221 The SVF can be isolated from either resected adipose tissue or aspirated adipose tissue using
16222 tumescent liposuction. Although a common procedure is still lacking, in general minced adipose tissue
16223 is digested by enzymes including collagenase, dispase, trypsin or the like [32]. However, mechanical
16224 procedures have also been reported [33]. After neutralising the enzymes, the released elements defined
16225 as SVF are separated from mature adipocytes by differential centrifugation.

16226 **32.10.4 Quality controls/release criteria**

16227 Currently, as there is no single marker to identify SVF cell subpopulations, the use of a
16228 combination of fluorochrome-labelled antibodies to surface antigens is suggested. The following
16229 markers or marker combinations should be considered for identifying the stromal cells within the SVF:
16230 $CD45^-/CD235a^-/CD31^-/CD34^+$, $CD13^+$, $CD73^+$, $CD90^+$, $CD105^+$. Alternative positive stromal cell
16231 markers, including CD10, CD29 and CD49, can be applied. Viability is recommended to be > 70 % to
16232 allow for good cell expansion. A fibroblastoid colony-formation unit assay and testing of the
16233 differentiation potential of the adipose stromal/stem cells within the SVF might enhance information on
16234 the quality of the cellular product [32].

16235 However, it is necessary to define and validate release criteria that are specific to the chosen
16236 clinical setting.

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32.10.5 Traceability

Records to ensure traceability, from the donation to the recipient, should be kept with the tissue establishment.

32.10.6 Biovigilance/pharmacovigilance

Although recent literature supports a paracrine role for SVF cells in regenerative settings, these same secreted cytokines may have adverse effects in the presence of tumour cells, e.g. recruiting the homing and promoting the proliferation of cancer cells following transplantation [32]. Hence, the safety of SVF treatment has to be evaluated carefully.

Table 32.1. Overview of developing cellular therapies: somatic cells used to restore or produce immunological functions in patients

	Starting material	*Processing steps*	*Parameters for specificity (quality control)*	*Storage*	*Transport/ distribution/ administration*	*Ref*
Antigen-specific T-cells (see §32.2)	Blood from heparinised sample or apheresis product Autologous or Allogeneic origin	***Selection of virus-specific or tumour-specific T-lymphocytes:*** 1. Direct T-cell selection from starting material based on i. phenotype with peptide-HLA-multimers *or* ii. Interferon gamma (IFNγ) production after stimulation with specific peptides and antigen-presenting cells with or without subsequent expansion by culture 2. Selection by *in vitro* culture	• Phenotype ($CD3^+$, $CD4^+$, $CD8^+$) • Specificity for the virus measured by • IFNγ production (ELISA or flow cytometry assay) • *or* • virus-directed lysis (chromium-release assay or flow cytometry assay)	≤ −140 °C	Administered fresh *or* Frozen vials thawed at bedside	1, 4
CAR-T-cells (see §32.2)	Blood from heparinised sample or apheresis product Autologous or Allogeneic origin	***Preparation of CAR-T-cells:*** 1. T-cell selection 2. Insertion of the modified gene for the chimeric receptor 3. *In vitro* expansion	• Phenotype ($CD3^+$, $CD4^+$, $CD8^+$) • Specificity for the antigen measured by • Expression and functionality of insert by cytokine production • Lysis of tumour cells by cytotoxicity assays (chromium-release assay or flow cytometry assays)	≤ −140 °C	Administered - immediately *or* Frozen vials thawed at bedside	2, 3
Natural killer cells (see §32.3)	Blood from heparinised sample, apheresis product or cord blood	***Selection of functional NK cells:*** 1. Obtain mononuclear fraction 2. Positive and/or cell selection using phenotypes: i. T-cell depletion (CD3) *and/or*	• Phenotype • Function measured by cytokine production and cytotoxicity	≤ −140 °C	Administered - immediately *or* Frozen vials thawed at bedside	7

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	Autologous or allogeneic origin	ii. NK cell enrichment (CD56) 3. (Optional) activation and expansion *in vitro*				
Dendritic cells (see §32.4)	Leukocytes collected by apheresis Autologous origin	***Isolation of monocytes:*** 1. Physical isolation (counterflow centrifuge elutriation) *or* Affinity column separation based on surface markers (CD14) 2. Culture with growth factors for differentiation into dendritic cells 3. Maturation step with required specificity	• Phenotype ($CD80^+$, CD83, $CD86^+$, $CD1a^+$, $HLA\text{-}DR^+$, $CD14^-$) • IL-12 production	≤−140 °C	Transport at low temperature Administration: immediate after thawing at bedside	9-11
MSC (see §32.5)	Bone marrow, peripheral blood, umbilical cord blood, adipose tissue, muscle, liver and umbilical cord tissue (Wharton's jelly) Allogeneic or autologous origin (depending on source)	***Generation of functional MSC:*** *In vitro* culture with growth factors (culture conditions depend on source of cells)	Phenotype: depends on the protocol for *in vitro* culture, but usually $CD73^+$, $CD90^+$ and $CD105^+$	≤−140 °C	Preparation of non-cryopreserved cells in syringe *or* Frozen vials thawed at bedside	10-13

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16251 16252 **Table 32.2. Overview of developing cellular therapies: somatic cells used to restore organ- or tissue-specific functions**

	Starting material	*Processing steps*	*Parameters for specificity (quality control)*	*Storage*	*Transport/ distribution/ administration*	*Ref*
MSC (see §32.6)	Bone marrow, peripheral blood, umbilical cord blood, adipose tissue, muscle, liver and umbilical cord tissue (Wharton's jelly) Allogeneic or autologous origin (depending on source)	***Generation of functional MSC:*** *In vitro* culture with growth factors (culture conditions depend on source of cells)	Phenotype: depends on the protocol for *in vitro* culture, but usually $CD73^+$, $CD90^+$ and $CD105^+$	≤ −140 °C	Preparation of non-cryopreserved cells in syringe *or* Frozen vials thawed at bedside	**10-13**
Chondrocytes (see §32.7)	Cartilage biopsy Autologous origin	***Isolation of chondrocytes:*** 1. Mechanical and enzymatic 2. Culture for 2-3 weeks as adherent cell layers (expansion) *and/or* Culture for 4-5 weeks expansion and attachment to scaffold for 3D support	• Morphology • Limited population doubling (max. 8×) • Purity of the population (mRNA)	Not to be stored	Chondrocytes are suspended into syringes/ ampoules for administration within 48 h	14-16
Keratinocytes (see §32.8)	Biopsy Autologous or allogeneic origin	***Isolation of -keratinocytes:*** 1. Mechanical *or* enzymatic in combination with mechanical 2. Culture with or without serum and feeder layer	• Morphology • Expansion rate	Ideally, use fresh. If necessary, store at ≤ −140 °C Short period of culture is needed after thawing, before distribution	Keratinocytes in sheets attached to synthetic carrier membrane and kept in culture media *or* Keratinocytes in suspension as spray – administration: immediate	17-22
Limbal cells (see §32.9)	Biopsy from the limbal region of the eye Autologous or allogeneic	***Generation of limbal cells:*** Biopsy is cultured on a feeder layer (irradiated) *or* Directly attached to amniotic membrane	• Morphology • Clonogenic assay (holoclones) • Transcription factors • Phenotypes for epithelial cells ($CK3^+$, $CK12^+$) The parameters assessed will depend on the culture system	No storage	2-8 °C during transport to the clinic Administration the same day as released	23-26, 28, 29

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Retinal pigment epithelial cells (see §32.9)	Embryonic stem cells, pluripotent stem cells, cord blood, fetal brain or bone marrow	***Generation of retinal pigment epithelial cells:*** *In vitro* culture with growth factors	• Immuno-histochemistry • Phagocytosis • Melanin content • Up-regulation of retinal pigment epithelial cells-specific genes	≤−140 °C	Thawed and re-suspended cells distributed to operating room at 2-8 °C	27
Corneal endothelial cells (see §32.9)	Corneo-scleral disc Allogeneic origin	***Isolation of corneal endothelial cells:*** 1. Mechanical, released during medium storage 2. Expanded *in vitro*	• Morphology • Expression of ZO-1 and Na^{+}/K^{+}-ATPase	No storage	Distributed to operating room at 2-8 °C	28
SVF (see §32.10)	Adipose tissue Autologous or allogeneic origin	***Isolation of SVF:*** Enzymatic and/or mechanical; no culture	• Phenotype (heterogeneous populations)	≤−140 °C	Administered immediately *or* Frozen vials thawed at bedside	30-33

MSC: mesenchymal stem cells. SVF: stromal vascular fraction.

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Chapter 33. Breast milk

33.1 Introduction

In the European Union (EU), human milk (HM) may fall within different national legal frameworks (see §33.1.1 below) for which appropriate quality and safety requirements need to be applied, e.g. food, tissues and cells [1]. It is essential that the ethical principles described in Chapter 1 of this Guide are respected so that donors are not exploited and the human body itself is not the subject of trade.

Regardless of the regulatory status of HM, ensuring safety and quality requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide. Thus, the guidance on selection and testing of donors, quality management and traceability described in the generic section (Part A) of this Guide can be applied usefully to the banking of HM to provide an appropriate framework for safe and effective services to patients.

HM contains essential nutrients and bioactive components that promote the growth and development of the newborn. HM not only covers the nutritional needs of healthy term infants but it also facilitates the process of maturation of various organs such as the gut and the brain. It is highly nutritious and contains a complex combination of immunological and anti-infective constituents that promote health, protect against infection and support a baby's immune system. For these reasons, a mother's own milk is universally accepted as the optimal feeding choice for neonates and infants but is considered as vital for preterm infants.

Unfortunately, not all preterm infants can receive milk from their mothers and some mothers are unable to provide enough milk for their infants. When this is the case, official bodies such as the World Health Organization (WHO), the American Academy of Pediatrics and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, as well as many scientific associations, consider donated human milk, obtained and processed in HM banks (HMB), to be the clinically preferred option in the absence of sufficient maternal milk [2, 3, 4, 5]. Donor HM should not be substituted for a mother's own milk if available. Given the significant impact that HMBs can have on infant health outcomes, the WHO has asked member countries "to promote the safe use of donor HM through HMBs for vulnerable infants" [2].

HMBs are institutions that collect, process, store and distribute HM prescribed for babies that are medically fragile, preterm babies, those with low birth weight, babies from mothers with delayed lactation, infants recovering from serious gut complications and surgery, and newborns affected by feeding intolerance, malabsorption syndromes or infectious diseases. The first HMB was established in Vienna in 1909. Since then, HMBs have been established in many countries: currently 224 HMBs exist in Europe [6], more than 300 in South America (217 in Brazil) and 26 in North America. The numbers and activities of HMBs are growing, driven by studies indicating that premature infants show better development if they are fed HM rather than formula. With donated HM, there is a lower rate of late-onset sepsis, a lower rate of necrotising enterocolitis and a better tolerance of enteral feeding, and better long-term psychomotor development [7].

In addition, it has been shown that where new HMBs are established there is an increase in the rates of breastfeeding on discharge from hospital of these babies [8-9]. It is now widely accepted that HMBs and the availability of donor HM encourage and support breastfeeding.

Theoretical microbiological risks associated with feeding with donor HM are similar to those in the food industry (i.e. contamination or cross-contamination with bacteria or fungi, with subsequent recipient infection). The food industry's standard quality assurance tool is HACCP (Hazard Analysis Critical Control Points) and it is widely recommended for HM banking. However, microbiological risks

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are also equivalent to those associated with transfusion and transplantation (i.e. transmission of viruses and other infective agents).

The following generic chapters (Part A) of this Guide all apply to milk banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, validation and risk management (Chapter 2);
c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Processing (Chapter 8);
g. Storage and release (Chapter 9);
h. Principles of microbiological control (Chapter 10);
i. Organisations responsible for human application (Chapter 12);
j. Computerised systems (Chapter 13);
k. Coding, packaging and labelling (Chapter 14);
l. Traceability (Chapter 15);
m. Biovigilance (Chapter 16).

33.1.1 Legal status of breast milk

Following a Competent Authorities expert group meeting on substances of human origin in June 2014, the European Commission addressed the questions related to the legal status of breast milk in the EU and, despite confirming that Article 168(4) of the Treaty on the Functioning of the European Union provides a legal basis for future regulation of these substances of human origin in terms of their quality and safety, it was clarified that member states are free to decide on the most suitable framework either by creating a specific regulatory framework at national level or by applying an existing national legislative framework, including the tissues and cells quality and safety requirements, to these substances [1].

33.2 Donor recruitment

The donation of HM must be voluntary and unpaid. In the case of reimbursement of expenses it should be in accordance with national regulation.

Promoting HM donation is carried out through a variety of different channels: written material (e.g. in prenatal clinics, paediatric surgeries, pharmacies, shops for maternity products), media, social networks, associations for breastfeeding programmes or educational guidance, and direct contact with pregnant women by their doctors and midwives.

A woman should be of legal age and lawfully competent to take this decision in accordance with national regulations: she should be nursing her own baby, who, if appropriate, should be adequately fed, before giving milk to a HMB. Bereaved mothers should be made aware of the possibility of donating their previously expressed and stored milk as well as continuing to lactate for the purposes of HM donation if desired. This has been shown to be of benefit to grieving mothers [10, 11].

Because HM donation is carried out frequently over a period of a few months, it must be stressed to the donor that certain health-related conditions – like infectious diseases or drugs (including nicotine and alcohol) – during the donation period would make her unsuitable as a HM donor. HMB staff have a duty of care to those who offer to donate HM, including a duty to those whose milk is not accepted because of, for example, medication or tobacco use. A mother who is unable to donate for whatever reason should be reassured that this should not affect her choice to feed her own baby – assuming that is the case. HMB staff should take responsibility for ensuring that she understands the reasons for her deferral and how this affects or does not affect her own infant [12]. The value of breast milk and of

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16417 breastfeeding her own infant in accordance with WHO guidance should be highlighted in all
16418 communications between HMB and prospective donors.

16419
33.3 Donor evaluation

16420 HMB processing cannot guarantee complete elimination of toxic substances and potential infectious
16421 elements that may be contained in the milk. For this reason, HM, which has not undergone any treatment,
16422 should be as safe as possible from the point of its origin.
16423 In addition to the general contraindications for donation specified in Chapter 4, the following
16424 conditions contraindicate the donation of milk [13, 14, 15, 16]:
16425 a. Donor's behavioural risks
16426 i. smoking tobacco, the use of snuff or use of nicotine-containing products to help stop smoking;
16427 wait for 7 days from the last exposure;
16428 ii. drug abuse or use of methadone;
16429 iii. daily consumption of beer (≥200 mL), wine (≥100 mL) or spirits (≥30-40 mL); occasional
16430 consumption may be accepted if milk collection is avoided for 12 hours;
16431 iv. consumption of high quantities (>300mg) of substances containing caffeine (coffee, tea, cola or
16432 cacao) should be avoided; occasional consumption may perhaps be accepted;
16433 v. if there is the suspicion of low vitamin B12 level (vegans or strict vegetarians, without vitamin
16434 B12 supplementation), the donor can be accepted if an adequate level of vitamin is verified in a
16435 blood test.
16436 b. Donor's treatments
16437 i. the use of drugs or other pharmacologically active substances (including herbal products) must be
16438 evaluated since most will be secreted into breast milk; the concentration and potential toxicity
16439 vary substantially depending on the substance and the dose (relevant information can be
16440 accessed at: www.e-lactancia.org/);
16441 ii. women immunised with attenuated live virus should not donate milk for 4 weeks after the
16442 immunisation;
16443 iii. the transfusion of blood and blood components, treatments with acupuncture needles that are
16444 not properly sterilised or disposable, endoscopic examinations or treatments made with flexible
16445 instruments (e.g. colonoscopy or bronchoscopy) and transplantation of organs, should exclude
16446 donation (organ transplantation is usually followed by long-term anti-rejection medication
16447 which excludes donation).
16448 c. Donor's medical history
16449 i. acute infections and diseases must be evaluated, depending on the type of infection for the
16450 appropriateness of temporary exclusion and the exclusion time itself;
16451 ii. women who have recently been in contact with infectious patients (e.g. chicken pox, mumps,
16452 measles) unless they have been immunised; if they have not been immunised, they should be
16453 excluded for a period equivalent to the incubation period or, if not known, for 4 weeks;
16454 iii. women with sexual contact with patients with viral hepatitis B or C should be excluded [13];
16455 iv. women with mastitis or fungal infections of the nipple or areola should be excluded temporarily;
16456 v. women with reactivation of *Herpes simplex* (HSV) or *varicella–zoster* infections in the
16457 mammary or thoracic region should be excluded;
16458 vi. women with a history of malignancy, including haematological malignancies, must be excluded
16459 since viruses have been shown to play a role in the development of some types of tumour.
16460 However, women with the following conditions can be accepted as donors:
16461 • cervical *in situ* carcinoma and localised skin tumours (basocellular carcinoma and squamous
16462 carcinoma) if they are removed and the donor has recovered;
16463 • some childhood solid tumours such as neuroblastoma, Wilms tumour and retinoblastoma; these
16464 are considered cured if the diagnosis was made before the donor was 5 years old and there has
16465 been no recurrence.

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33.4 Milk donor testing

In addition to the tests outlined in Chapter 5, testing for HTLV I/II is recommended in most of the guidelines for HMBs; at least it should certainly be performed for donors living in or originating from high-prevalence areas or with known sexual partners originating from those areas, or if the donor's parents originate from those areas.

The risk of transmission of *Trypanosoma Cruzi* through breastfeeding has not clearly been established. Pasteurisation destroys *T Cruzi* [14]. If there is a suspicion of this disease during the health interview, serological screening for *T. Cruzi* should be done.

Theoretically, it is not necessary to repeat donor testing during the period of milk donation if there is no change to the donor's risk status, When careful evaluation of the change in donor's risk status is not feasible in practice, donors should be tested every 3 months.

Seropositivity for *Cytomegalovirus* (CMV) is not considered a contraindication as long as the milk is pasteurised.

33.5 Procurement

The milk can be expressed by hand or with a breast pump. Good breast hygiene should be encouraged and at least once daily washing of the breasts is recommended. Additional washing prior to expressing has also been shown to reduce bacterial contamination of the milk, together with cleaning and disinfecting all the components of breast pumps [16].

Most recommended containers are rigid plastic, suitable for food use and made from a variety of materials such as polyethylene or polypropylene. It is recommended that containers should be sterile and single-use. Re-using containers requires cleaning and disinfection. The use of containers sterilised with ethylene oxide is regulated at the European level, and users should ascertain that manufacturers respect this EU regulation (or users should avoid devices sterilised with ethylene oxide, as shown on the label). Glass containers can be used; however, they should be purpose-designed and sold as intended for breast milk storage, including freezing and high temperatures, and made from glass that is chosen to resist breakage [17].

Some milk banks use plastic bags of polyethylene as an alternative to rigid containers; but these bags can easily rupture with the risk of loss of milk and contamination. Use of a double bag is therefore recommended if bags are used.

If the milk is to be frozen, the container should not be filled completely. The containers must be labelled with a donor code (or donor's given name and family name), and the date of collection.

33.6 Temporary milk storage and transportation to the milk bank

Collected milk should be kept at room temperature for the shortest possible time. After collection, the container should be sealed and cooled in order to avoid bacterial growth and degradation of the milk [10, 18].

It can be stored at 4 °C for 24 hours and then frozen at ≤−20 °C. Some HMB accept the pooling of milk of different collections from the same mother as long as the milk is kept in the fridge, but the new milk should first be cooled before being added to previously collected milk [19, 20].

If a woman has accumulated milk before being accepted as a donor, medical and behavioural history must be evaluated retrospectively for suitability, and the milk can be accepted only if it has been appropriately preserved and identified.

HM for donation should be transported frozen to the milk bank. Dry ice may be used as the refrigerant during transport; however, frozen cool packs to fill any spaces within the insulated transport containers are generally sufficient for short journeys. It is recommended that the milk bank is responsible for the transportation of milk. If third parties are used, there must be a formal agreement in place, with the milk bank covering transport conditions to ensure the safety and quality of the milk.

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The transport process must be validated or temperatures monitored during transport to ensure the milk is kept under appropriate conditions.

Evidence of the integrity of the containers on arrival at the milk bank must be documented.

33.7 Processing

It is common practice in HMBs to increase safety and reduce the risk of contamination by pasteurising the milk.

However, in Norway raw (i.e. non-pasteurised) milk from CMV-negative donors is used in very specific contexts [21], and the donors who are suitable for milk banks are not a high-risk group for any of the viral diseases screened before donation (HIV, hepatitis, HTLV etc.). Furthermore, in Norway there is bacteriological screening of all donated milk, and samples with a bacterial count of more than 10^4 and less than 10^5 colony-forming units/mL are pasteurised [22]. However, the use of raw donor HM in Norway is constantly under review [21].

Recent guidelines for pasteurisation recommend a temperature of 62.5 °C for 30 minutes, the so-called Holder pasteurisation [23]. It is recommended that the heated milk should be cooled to 25 °C within 10 minutes, although a final temperature of 10 °C or lower is preferable [17].

It has been shown that pasteurisation does not affect the macronutrient composition (protein, carbohydrates and lipids, including polyunsaturated fatty acids) of milk and that HM oligosaccharides are preserved by freezing and pasteurisation. However, new treatments are under development to provide the same level of safety without affecting the other biological components of HM [23, 24, 25, 26, 27),

The pasteurisation process begins with the thawing of milk either slowly, overnight in a refrigerator, or quickly by immersion in a water bath with stirring at a controlled temperature, not higher than 37 °C [13]. The milk should be refrigerated as soon as it has thawed, to prevent bacterial proliferation, and pasteurised within 24 hours of thawing.

Milk must be processed under hygienic conditions. Individuals handling open containers of milk must wear a hair covering, gloves and a clean gown, apron or lab coat to prevent contamination of the milk. Although it may not be considered strictly necessary, an increasing number of HMBs handle the milk in a laminar-flow or bio-safety cabinet, which must be qualified (including regular analyses of the particulate and microbiological contamination of the cabinet).

After thawing, some HMBs combine or pool milk from multiple donors. This practice of pooling may increase uniformity in the product and provide more consistent nutrient content; however, if there is contamination of pooled milk, it may be difficult to trace the source of the contamination. If milk pooling is practised, the HMB must decide whether pooling will be allowed between different donors, and the maximum number of donors whose milk may be pooled.

Before pasteurisation, a sample of milk from each batch should be taken for microbiological testing. This sample may also be used for a macronutrient analysis. The pasteurisation process can be carried out in a shaker water bath, or equipment specifically designed for pasteurising milk may be used. The equipment used for pasteurisation must be calibrated at least every 12 months [6]. Regular qualification of pasteurisers is needed to optimise pasteurisation and milk quality. A few criteria have been proposed: temperature plateau of 62.5-64.5°C, duration of the plateau between 30 and 35 minutes, exposure time over 58 °C $<$ 50 min and exposure time from 62.5 °C to 6 °C $\leq$ 1h [26, 27].

During pasteurisation, bottle caps must remain above water level to prevent contamination, unless caps and equipment designed for submersion are used, in which case additional checks should be in place to ensure seals are effective and end users should be cautioned to discard containers with an incomplete seal and to notify the milk bank immediately. A control bottle containing the same amount of milk or water as the fullest container of milk in the batch shall be fitted with a calibrated thermometer to record milk temperature during pasteurisation. The control bottle should follow the same process as the rest of the batch at all times. In addition to the milk temperature, the water bath temperature must be monitored and recorded.

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16561 At the end of the process, a sample of the pasteurised milk should be taken for microbiological
16562 testing. It is advisable to keep a sample of each batch of pasteurised milk for further tests should the
16563 need arise.

16564 33.8 Quality control

16565 The first control for donated milk, before processing, is the evaluation of its appearance. Human milk
16566 varies widely in colour and may be clear (early colostrum), yellow (colostrum and early transitional
16567 milk), creamy white and white tinged with blue or green, depending on the age of the baby, the extent
16568 to which the breast has been emptied and diet. If the milk exhibits unusual colouring or if it contains
16569 any foreign bodies or visible impurities, these should be noted. This information may help final decision
16570 making. If it contains foreign bodies, it should be discarded.

16571 33.8.1 Microbiological evaluation

16572 The microbiological evaluation of donated HM includes the testing of each batch before and after,
16573 to identify unusual or heavy contamination and pathogens. However, there is no consensus within
16574 internationally published guidelines of the optimal microbiological control both before and after
16575 pasteurisation [6, 7, 9]. A similar level of discrepancy is observed in the criteria of acceptability of the
16576 milk when bacteriologic analysis is performed, as is shown in Table 33.1.The criterion for discarding
16577 pasteurised milk should be any microbial growth.

16578 33.9 Labelling and packaging

16579 In addition to the information about labelling set out in Chapter 14, labels for packaging pasteurised milk
16580 may contain information about the nutritional value, such as the concentrations of protein, fats and
16581 carbohydrates, and the energy content.
16582 The volumes of the final storage containers may vary depending on the needs of the recipient.
16583 The most common volumes are 50, 100 and 200 mL.

16584 33.10 Preservation/storage

16585 The most common method of milk preservation is freezing ≤−20 °C. Devices used for freezing should
16586 be qualified and the temperature recorded and controlled using calibrated probes.
16587 Milk that has not been frozen is acceptable for use (or for freezing) only if kept for less than 24
16588 hours from collection.
16589 Despite its advantages, very few milk banks use lyophilisation after pasteurisation as a method of
16590 preservation. Donor milk that has been lyophilised after pasteurisation can be stored at ambient
16591 temperature, as a powder, for up to 18 months, versus 6 months after pasteurisation alone. It is used to
16592 supply donor human milk to French overseas territories. However, the HMB of Bordeaux-Marmande
16593 uses Holder pasteurisation followed by lyophilisation and processes more than 12 000 litres of milk per
16594 year [28].

16595 33.10.1 Expiry date

16596 There is no unanimous agreement about the expiry date for milk. In most European countries, it
16597 is accepted that milk should not be kept more than 4 months at −20 °C before pasteurisation whereas the
16598 USA and Canada accept storage for up to 12 months. After processing, milk may be stored for between
16599 3 and 12 months, depending on the country [17].
16600 In the case of milk stored at −80 °C, no more than 12 months' storage both before and after
16601 pasteurisation is the recommended maximum [16].
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Table 33.1. Criteria for the discard of milk before pasteurisation

NICE [UK] Guidelines	Total bacteria $> 10^5$ CFU/mL	*Enterobacteriaceae*$> 10^4$ CFU/mL	*Staphylococcus aureus*$> 10^4$ CFU/mL
Italian guidelines	Total bacteria $> 10^5$ CFU/mL	*Enterobacteriaceae*$> 10^4$ CFU/mL	*Staphylococcus aureus*$> 10^4$ CFU/mL
French legislation	Total (aerobic) flora $> 10^6$ CFU/mL		*Staphylococcus aureus*$> 10^4$ CFU/mL
Australian guidelines	Confluent bacterial growth $> 10^5$ CFU/mL	Any *enterobacteriaceae*, *enterococci* or potential pathogens capable of producing heat-stable enterotoxins	
Indian guidelines			*Staphylococcus aureus*$> 10^4$ CFU/mL
Swedish guidelines	Total bacteria $> 10^7$ CFU/mL	*Enterobacteriaceae*$> 10^7$ CFU/mL Any pathogenic bacteria	*Staphylococcus aureus*$> 10^7$ CFU/mL
North American guidelines	[no testing]		

33.10.2 Storage temperature

According to the basic principles of freezing, frozen foods at −18 °C are safe indefinitely from bacterial contamination, although enzymatic processes inherent in food could persist, with possible changes in milk quality [29]. Freezing milk at − 20 °C/− 30 °C before and after processing slows down but does not stop lipolysis. However, it has been recently reported that storage of human milk at −20 °C for 9 months was associated with preservation of key macronutrients and immunoactive components [30].

When human milk is preserved at −80 °C, lipolysis is stopped. Long-term storage at −80 °C has been proposed but it has been recently reported that freezing at these temperatures significantly decreases the energy content of HM, both from fat and carbohydrates [20, 28, 32].

For pasteurised donor human milk, most guidelines recommend storage in freezers at −18 °C to −20 °C, for a maximum duration of 3 to 6 months [33, 34, 35, 36].

33.11 Distribution and transport conditions

Distribution of milk must be so conducted as to ensure product traceability between donor and recipient, as described in Chapter 15 of this Guide. The HMB must keep records of the documentation of each donor, the processing pool, qualification, storage and final destination (distribution, disposal, expiry date), and the hospital must document how the milk is used.

During transport, milk must remain frozen, and dry ice may be used for this purpose. The freezing point of milk is at a lower temperature than that of water and, consequently, use of water ice as a coolant during transportation can result in partial melting of the milk. The use of validated, easily cleaned insulated transport containers is recommended.

The transport procedure should be validated, and the temperature of the transport container should be monitored at all times during transportation. In the case of transport by third parties, a formal agreement is required with the milk bank to ensure appropriate transport conditions are maintained.

33.12 Biovigilance

As described in Chapter 16, deviations from the standard operating procedure (SOP), from donation to the administration of human milk, should be recorded and documented, as well as adverse reactions after application [37].

In addition to milk banks, hospitals should also have appropriate SOPs for the storage, thawing and handling of milk containers to avoid degradation of the quality of the milk and the possibility of adverse reactions in recipients.

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16636 Milk should not be thawed in a microwave oven, since that significantly reduces the amount of
16637 vitamin C, the total IgA content and lysozyme activity [38].
16638 Milk thawed in the refrigerator can be kept at 4 °C for up to 72 hours if the container has not been
16639 opened. Once opened, the package should be consumed within 30 hours. Furthermore, thawed milk must
16640 not be re-frozen.

16641 33.13 New techniques for processing

16642 Several techniques have been investigated to eliminate pathogens in milk without affecting its biological
16643 properties. These include:
16644 • high temperature short time (HTST) or ultra-high short time (UHST),
16645 • ultra-pasteurisation or ultra-high temperature (UHT).

16646 There are also methods for reducing micro-organisms in food that do not use heat. While not
16647 technically pasteurisation, they achieve the same effect and are known as cold pasteurisation. These
16648 include:
16649 • high-pressure processing (HPP) or pascalisation,
16650 • ultraviolet (UV) irradiation,
16651 • ultrasonication,
16652 • high intensity pulsed electric field (PEF).

16653 Even if such techniques are shown to be effective and preserve important bioactive components
16654 of HM better than Holder pasteurisation, they may be difficult to translate into practice, given the lack
16655 of appropriately scaled equipment for use in HMBs. Furthermore, these developing devices need to be
16656 validated in real conditions, with milk volumes that are currently treated in milk banks, and to be
16657 compared with Holder pasteurisation performed with qualified pasteurisers. When testing new
16658 technologies, precise description of the process and recording of the process parameters are necessary.

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Chapter 34. Faecal Microbiota

34.1. General considerations

The regulatory classification of faecal microbiota is challenging because they do not fit clearly into one group of therapies or another, whether the criterion applied is anatomical origin, method of application, mode of action or complexity of processing.

Hence, a wide variety of approaches to regulation exist in Europe, and in some cases faecal microbiota are banked outside any regulatory framework. Following a Competent Authorities expert group meeting on substances of human origin in June 2014, the European Commission addressed the questions related to the legal status of faecal microbiota in the EU and, despite confirming that Article 168 (4) of the Treaty on the Functioning of the European Union provides a legal basis for future regulation of these substances of human origin in terms of their quality and safety, it was clarified that member states are free to decide on the most suitable framework (e.g. medicinal products, tissues and cells) either by creating a specific regulatory framework at national level or by applying an existing national legislative framework, including the tissues and cells quality and safety requirements, to these substances. Several countries have introduced some national rules and others require compliance with Directive 2004/23/EC on the quality and safety of tissues and cells (see also §34.3 below).

Given the significant commercial interest in developing these services on a for-profit basis, it is essential that the ethical principles described in Chapter 1 of this Guide are respected so that donors are not exploited and the human body itself is not the subject of trade. Promotion of altruistic unpaid donation of faecal microbiota by means of advertisement or public appeal may be undertaken in accordance with domestic regulations.

Regardless of the regulatory status of faecal microbiota, ensuring their safety and quality requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide. Thus, the guidance on selection and testing of donors, quality management and traceability described in the generic section (Part A) of this Guide can be applied usefully to the banking of faecal microbiota to provide an appropriate framework for safe and effective services to patients.

34.2.Introduction

Faecal microbiota transplantation (FMT) is the transfer of biological material containing a minimally manipulated community of micro-organisms from a human donor to a human recipient (including autologous use) with the intent to restore the diversity of gut microflora. FMT may confer protection against toxigenic *Clostridium difficile* [1, 2]. FMT was first reported in 1958, by Eiseman *et al., to treat a case of pseudomembranous colitis* [3]. Since then, a large body of evidence, including randomised controlled trials, systematic reviews and meta-analyses, has proved clear evidence that FMT is a highly effective treatment against recurrent *Clostridium difficile* infection unresponsive to repeated antibiotic treatments (rCDI) [4-10]. Due to the rising prevalence, severity and mortality of this infection, the therapeutic role played by FMT is therefore important to save human lives and to decrease the economic burden on healthcare systems [11-14]. Based on these data, both the European Society for Microbiology and Infectious Disease and the American College of Gastroenterology recommend FMT as a treatment for recurrent *Clostridium difficile* infection [15, 16]. A 2017 European consensus conference report strongly recommends the implementation of FMT centres for the treatment of *Clostridium difficile* infection [17].

FMT has also been investigated in the treatment of other disorders associated with the alteration of gut microbiota. In particular, studies in humans include randomised controlled trials [18-21] with

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systematic review and meta-analysis, in patients with IBD, especially ulcerative colitis UC [22-24], and case series reports in patients with metabolic syndrome [25, 26], hepatic encephalopathy [27] and graft *versus* host disease [28].

The following generic chapters (Part A) of this Guide all apply to FMT and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management, risk management and validation (Chapter 2);
c. Recruitment of potential donors, identification and consent (Chapter 3);
d. Donor evaluation (Chapter 4);
e. Donor testing (Chapter 5);
f. Procurement (Chapter 6);
g. Premises (Chapter 7);
h. Processing (Chapter 8);
i. Storage and release (Chapter 9);
j. Principles of microbiological testing (Chapter 10);
k. Distribution and import/export (Chapter 11);
l. Organisations responsible for human application (Chapter 12);
m. Computerised systems (Chapter 13);
n. Coding, labelling and packaging (Chapter 14);
o. Traceability (Chapter 15);
p. Biovigilance (Chapter 16).

34.3. Donor evaluation – exclusion criteria

Limited trials have evaluated the outcomes of FMT as related to donor or FM characteristics (see §34.10). Current criteria are based on expert opinion, guidelines and rules from other domains (e.g. blood donation) [29, 30]. Published results from studies demonstrate that limited percentages of donors screened met the criteria for donation [31-34].

The selection of donors for FMT has two main aims. The first aim is to prevent adverse events potentially associated with the infusion of faecal material, while the second aim is to avoid the transmission of impaired microbiota that could be not useful (or harmful) to the recipient.

According to the recommendations of the European FMT Working Group [17], potential donors should undergo four different steps to be selected, as follows:

a. written questionnaire;
b. general clinical examination;
c. blood and stool testing;
d. further questionnaire (the day of the donation).

First, potential donors should complete a written questionnaire to assess their medical history and lifestyle habits. This approach is particularly important to rule out issues not detectable by laboratory testing.

Usually subjects younger than 60 years old are preferred, as older individuals are more likely to suffer from other diseases. However, this suggestion should not be mandatory.

The questionnaire should be designed both to exclude the risk factors for infectious diseases, as required by the European Commission to select allogeneic living donors of human tissue transplants (Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells) and to identify subjects who have gastrointestinal (GI) disorders or who take drugs, which can alter the donor microbiota [35, 36] (see Table 34.1).

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16835 **Table 34.1. Exclusion criteria for stool donors to be addressed in the written questionnaire**

- history of, or known exposure to, HIV, HBV or HCV, syphilis, human T-lymphotropic virus I and II, malaria, trypanosomiasis, tuberculosis
- known systemic infection not controlled at the time of donation
- use of illegal drugs
- risky sexual behaviour (anonymous sexual contacts; sexual contacts with prostitutes, drug addicts, individuals with HIV, viral hepatitis, syphilis; work as prostitute; history of sexually transmittable disease)
- previous reception of tissue/organ transplant
- previous (<12 months) reception of blood products
- recent (<6 months) needle stick accident
- recent (<6 months) body tattoo, piercing, earring, acupuncture
- recent medical treatment in poor hygienic conditions
- risk of transmission of diseases caused by prions
- recent parasitosis or infection from rotavirus, *Giardia lamblia* and other microbes with GI involvement
- recent (<6 months) travel in tropical countries, countries at high risk of communicable diseases or traveller's diarrhoea
- recent (<6 months) history of vaccination with a live attenuated virus, if there is a possible risk of transmission
- healthcare workers (to exclude the risk of transmission of multidrug-resistant organisms)
- individual working with animals (to exclude the risk of transmission of zoonotic infections)
- history of IBS, IBD, functional chronic constipation, coeliac disease, other chronic GI disorders
- history of chronic, systemic autoimmune disorders with GI involvement
- history of, or high risk for, GI cancer or polyposis
- recent appearance of diarrhoea, haematochezia
- history of neurological/neurodegenerative disorders
- history of psychiatric conditions
- overweight and obesity (body mass index >25)
- recent (<3 months) exposure to antibiotics, immunosuppressants, chemotherapy
- chronic therapy with proton pump inhibitors

Source: European FMT Working Group consensus [17]

16836
16837 Candidates who are suitable for donation on the basis of the questionnaire should then undergo
16838 blood and stool testing (Table 34.2), which should be done no longer than eight weeks before donation
16839 [17]; after this term, testing exams should be repeated before further donations. The tests should be
16840 validated [29, 37] and carried out in accordance with the specification in Chapter 5.
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16843 **Table 34.2. Blood and stool testing for donor selection**

General blood testing

- Cytomegalovirus
- Epstein-Barr virus
- hepatitis A
- HBV
- HCV
- hepatitis E virus
- syphilis
- HIV-1 and HIV-2
- *Entamoeba histolytica*
- complete blood cell count with differential
- C-reactive protein and erythrocyte sedimentation rate
- albumin
- creatinine and electrolytes
- aminotransferases, bilirubin, gamma-glutamyltransferase, alkaline phosphatase

Blood testing to be performed in specific situations

- human T-lymphotropic virus types I and II antibodies
- Strongyloides stercoralis

General stool testing

- detection of *Clostridium difficile*
- detection of enteric pathogens, including *Salmonella, Shigella*
- *Campylobacter, Escherichia coli* O157 H7, *Yersinia*, vancomycin-resistant *Enterococci*, methicillin-resistant *Staphylococcus aureus*
- Gram-negative multidrug-resistant bacteria
- norovirus
- antigens and/or acid fast staining for *Giardia lamblia* and *Criptosporidium parvum*
- protozoa (including *Blastocystis hominis*) and helminths
- faecal occult blood testing

Stool testing to be performed in specific situations

- detection of *Vibrio cholera* and *Listeria monocytogenes*
- antigens and/or acid fast staining for *Isospora* and *Microsporidia*
- calprotectin
- *Helicobacter pylori* faecal antigen
- rotavirus

Source: European FMT Working Group consensus [17]

16844
16845 Finally, the last assessment of selected donors on the day of the donation should be done by
16846 questionnaire to rule out any newly onset issue that could impair the donation (see Table 34.3). If the
16847 centre has been authorised for (or has the expertise in) the management of frozen stools, blood tests for
16848 HBV, HCV and HIV should be repeated before storage in order to check for possible infections occurring
16849 in the window phase (nucleic acid testing assays should be performed due to the substantial decrease in
16850 window period comparing with routine serological tests).
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16853 **Table 34.3. Issues to be addressed by questionnaire the day of the donation**

• newly appeared GI signs and symptoms (e.g. diarrhoea, nausea, vomiting, abdominal pain) • newly appeared illness or general signs (e.g. fever, throat pain, swollen lymph nodes) • use of antibiotics or other drugs that may impair gut microbiota, new sexual partners or travels abroad since the last screening • recent ingestion of a substance that may result in harm for the recipients • travel in tropical areas • contact with human blood (sting, wound, showing, piercings, tattoos) • sexual high-risk behaviour • diarrhoea (more than three loose or liquid stools per day) among members of the entourage (including children) within 4 weeks of donation
Source: European FMT Working Group consensus [17]

16854
16855 Current evidence does not support the superiority of related donors over unrelated ones, at least
16856 when FMT is administered to cure *C. difficile* infection [38]. For other indications, definitive data are
16857 still not available. The use of anonymous healthy donors may be useful in large centres to allow
16858 clinicians to satisfy the need for FMT to treat *C. difficile* infection. The recruitment of potentially
16859 interested donors could be organised through existing pathways (e.g. blood donors). The procurement
16860 must be authorised only after informed consent procedure. According to the European directives
16861 mentioned above, donation is voluntary and unpaid, a factor which may contribute to high safety
16862 standards and therefore to the protection of human health [35].

16863 34.4.Preparation of donors

16864 There is no specific preparation of the donor. The donor can be advised to avoid food that can be allergic
16865 for the recipient, such as peanuts, other nuts and shellfish within the 5 days prior to donation. Consider
16866 the use of a gentle osmotic laxative the night before donation [38, 39].

16867 34.5.Procurement, transportation to the processing facility, processing
16868 of faeces and storage

16869 Faeces will, most likely, be collected by the donor at home. The travel distance to the processing facility
16870 is important as it is generally believed that a high viability of bacteria in stools increases the chance of
16871 successful FMT. The processing facility should provide sterile faecal containers in order to prevent
16872 contamination.
16873 The data related to the donation shall be handled with respect for confidentiality of any health-
16874 related information provided to the authorised personnel, the results of tests on their donations, and
16875 details of traceability from donor to recipient and vice versa.
16876 Faeces have traditionally been processed for immediate 'fresh' use. More recently evidence has
16877 accumulated that the use of frozen FMT is as effective as fresh FMT. Frozen FMT has different
16878 advantages, mainly from the logistical point of view (selection and screening of donor, quality of stool
16879 etc.) [6, 40, 41].

16880 34.5.1. Stool handling and fresh faeces preparation

16881 To protect anaerobic bacteria, the storage and preparation should be as brief as possible. The stool
16882 should be processed, following safety requirements, in Class II biosafety cabinets. Protective gloves and
16883 facial masks should be used during preparation. Until further processing, the stool sample can be stored
16884 at ambient temperature. 'Ambient' is rather ill-defined and comparable to 'room temperature'. A
16885 minimum amount of 30-50 g of faeces should be used [10, 42-44]. Anaerobic storage and processing
16886 should be applied if possible; and a dedicated space, disinfected using measures that are effective against
16887 sporulating bacteria, should be used. Faecal material should be suspended in saline [45], using a blender

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or manual effort, and sieved in order to avoid the clogging of infusion syringes and tubes. Fresh stool should be processed and used preferably within 6 hours after donation [4, 6, 10, 38, 41, 42].

34.5.2. Preparation and defrosting of frozen faecal material

At least 30 g of donor faeces and 150 mL of saline solution should be used. Similarly to fresh samples, preparation of frozen faecal suspensions under normal air or under oxygen-free atmosphere yielded a similar resolution rate [6, 42, 46]. Before freezing, glycerol should be added up to a final concentration of 10 % [47, 52]. Inulin would seem to be an equivalent alternative for glycerol [48]. The final suspension should be clearly labelled and traceable, and stored at –80 °C. The labels should include a unique donor code, suspension number, production and expiration date, volume and storage instructions.

The frozen faecal material should be stored in dedicated freezers since the faecal material in itself is contaminated and further (cross-)contamination should be avoided. Information regarding length of storage is limited: 5 to 6 months should be acceptable, but probably longer too. On the day of faecal infusion, the faecal suspension should be thawed in a warm (37 °C) water bath and infused within 6 hours from thawing. After thawing, saline solution can be added to obtain a desired suspension volume. Since microbial cells are sensitive after defrosting, repeated thawing and freezing should be avoided [49].

34.5.3. Quality control

Samples of donor faeces before the processing and before the administration to recipients should be stored for possible microbiological evaluation and qualitative and quantitative characterisation for safety reasons. The frozen samples should be stored in adequate facilities (or, when specifically regulated, by authorised facilities) and should be clearly labelled with the code of the donor and the date of donation.

34.6. Basic requirements for implementing an FMT centre

Stool banking and centres for the treatment of *Clostridium difficile* infection should be implemented in hospitals with appropriate expertise and facilities [17]. The dissemination of the FMT procedure and the establishment of FMT services in clinical settings could be useful practices to reduce the *Clostridium difficile*-related healthcare burden [44, 50-54]. The development of an FMT centre service would ensure the optimal standardisation of the FMT process. The availability of several facilities (including endoscopy service, clinical ward and outpatient clinic) is essential to implement an FMT centre. FMT can be performed in either an inpatient or an outpatient setting.

FMT centres need to have access to, or be part of, a facility that allows safe processing of human samples (biosafety level 2) including aliquoting, storage and preparation of faeces [55]. In fact, one of the key functions of the FMT centre is the management of blood and faecal samples from either donor or recipients. *C. difficile* is a pathogen with a biosafety level 2 (cabinet with high-efficiency particulate air filter) and safety requirements and recommendations need to be implemented accordingly [55]. Material processing (fresh faeces, banks of frozen and stored faeces) and safety precautions have to adhere to basic principles for safe preparation of human material, including: rigorous protocols in securing the materials; maintenance of standard operating procedures for the processing; use of certified laboratory testing; definition of quality-control tests and standards for the release of the final product.

Finally, management of the records related to the FMT procedure should be regulated by a local health organisation. FMT procedure and donors' and recipients' records should be stored for at least 10 years. According to Directive 2004/23/EC, data required for full traceability must be kept for a minimum of 30 years after clinical use [35]. This may differ from local requirements, and longer storage time could be needed. The records of the FMT centre will provide access to the long-term safety data.

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34.7. Preparation of recipients

Patients with *C. difficile* infection should be pre-treated with vancomycin or fidaxomicin, at least for 3 days and until 12–48 hours before FMT. This pre-treatment aims both to reduce bowel movements (allowing a longer persistence of the infusate in the bowel of the recipient) and to provide a bridging therapy to recipients while they are waiting for the procedure. In case of emergency, antibiotic pre-treatment can be avoided if donor samples are quickly available. For other indications beyond *C. difficile* infection, there is no reliable evidence to recommend any pre-treatment before FMT [17].

However, recipients should undergo bowel lavage by polyethylene glycol before FMT, at least when it is administered by upper route or by colonoscopy [17], to reduce the *C. difficile* load in the intestines. Inadequate bowel preparation has been identified as a risk factor for failure of FMT [56]. However, current evidence of enema FMT shows high cure rates without bowel lavage before the infusion [6], so no indications for bowel preparation have been suggested for this route of delivery.

34.8. Delivery of faecal material

FMT can be performed through different routes of delivery, including colonoscopy, upper endoscopy, nasoduodenal/nasojejunal tube, enema or capsule. For each route of delivery, faecal infusions can be repeated if a single one fails to cure *C. difficile* infection. Risk factors for failure of a single faecal infusion include inadequate bowel preparation, severe *C. difficile* infection [56] and hospitalisation during FMT [57].

Several systematic reviews and meta-analyses found that colonoscopy provided higher resolution rates of *C. difficile* infection than other routes and a similar safety profile [7-9, 58].

During colonoscopy, the faecal material should be administered into the right colon of the recipient, when possible. In patients with severe *C. difficile* infection, it can also be infused in the left colon, for safety reasons.

FMT via enema showed satisfactory results in treating *C. difficile* infection, and repeated infusions could increase its success rates [6]. Enema may be useful for several reasons, including poor invasivity and costs, and wide availability.

Moreover, before starting the infusion through upper routes, by gastroscopy, by gastroduodenal or nasojejunal tube, or by gastrostomy tube, recipients must be positioned 45° upright, and keep this position for at least 4 hours after it, to reduce the risk of aspiration [17].

The ideal volume for instillation has not been established. However, smaller volumes (e.g. 25-50 mL) could be used for delivery via a nasoduodenal tube or nasogastric intubation; larger volumes (e.g. 250-500 mL) could be used for instillation via colonoscopy [41, 42].

34.9. Monitoring of patients in biovigilance

Recipients should be monitored for the occurrence of possible acute complications related to the procedure. Infection-control practices for patients with rCDI should be performed according to disease severity and comorbidities. The need for hospitalisation of patients with other underlying diseases depends on the diagnosis and clinical condition. When repeated faecal infusions are necessary, provided that the patient's condition is good, further applications can be performed in an outpatient setting [17]. The duration of the observation period has not been defined yet, as it depends on the route of delivery, the underlying diseases and the general condition of the patient.

Periodicity and length of follow-up for long-term adverse events and reactions are not determined. Follow-up should include clinical and analytical data.

Adverse events and reactions are not rare and should be carefully monitored throughout FMT [59]. The vast majority are mild, self-limiting and gastrointestinal in nature [60]. However, severe adverse reactions (such as death, viral and bacterial infections, transient relapse of IBD), were reported

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in several studies [59], but in a number of cases the association with FMT was not established due to the lack of controlled data.

These findings mandate the creation of registries – at the local (hospital) level and/or at the level of regional, national or international competent authorities – recording well-defined and standardised data. In order to trace possible causality linking FMT and newly developed diseases, keeping appropriate registries would be a wise process to trace and learn about potential long-term side effects.

34.10. Recent developments

FMT has also been investigated in the treatment of other disorders associated with the alteration of gut microbiota. In particular, studies in humans include ulcerative colitis [18-24], patients with metabolic syndrome [25, 26] or hepatic encephalopathy [27] and graft *versus* host disease [28]. FMT has been also thought to treat various diseases, including Parkinson, multiple sclerosis, fibromyalgia and chronic fatigue syndrome, among others [17, 61]. England's National Institute for Health and Care Excellence (NICE) has also published guidance on interventional procedures using FMT [62].

More recently, the composition of the intestinal microbiota has been thoroughly investigated and shown to vary among individuals and throughout development, and to be dependent on host and environmental factors [63-65]. These results are an important step towards better understanding of environment–diet–microbe–host interactions and further understanding of the role of dysbiosis, the role of FMT as a therapy and the importance of donor selection in clinical success [66, 67].

Nowadays, accumulating evidence is elucidating the relation of dysbiosis of intestinal bacteria with obesity and metabolic disorders. Certain gut microbial strains have been shown to inhibit or attenuate immune responses related to chronic inflammation in experimental models, suggesting that specific species among gut microbiota may play either a protective or a pathogenic role in the progression of obesity [68].

Moreover, capsule FMT represents a new but promising approach [69-72], which may increase the availability of FMT, both by expanding the access to treatment and by ameliorating the patient compliance to this procedure. Similarly lyophilisation (freeze-drying) could simplify FMT treatment [40, 73].

Finally, an NIH-funded study, aimed at addressing the regulation of (F)MT, adopted the following definition of MT:

> A microbiota transplantation is the transfer of biological material containing a minimally manipulated community of microorganisms from a human donor to a human recipient (including autologous use) with the intent of affecting the microbiota of the recipient. [74]

Minimal manipulation is processing that does not alter the original relevant characteristics of the transferred community of micro-organisms.

34.11. References

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Chapter 35 Serum eye drops and platelet derivatives

35.1. Introduction

Serum eye drops and platelet derivates are examples of substances of human origin where there is wide variation in approach to regulation in Europe [1]. In some circumstances, similar substances can be banked outside any regulatory framework.

Within the European Union (EU), blood used for the manufacture of serum eye drops must meet the standards of quality and safety specified in Commission Directive 2004/33/EC of 22 March 2004, which implements Directive 2002/98/EC of the European Parliament and of the Council regarding certain technical requirements for blood and blood components, including donor selection. For cord blood serum, the selection criteria for living donors of tissues and cells specified in Annex I/III of Directive 2006/17/EC are applicable for EU member states. Serum eye drops per se and platelet derivates may fall within different national legal frameworks in the EU for which the appropriate quality, safety, and vigilance requirements need to be applied (these may include blood, tissues and cells, medicinal products).

The following generic chapters (Part A) of this Guide all apply to serum eye drops and must be read in conjunction with this chapter where applicable:

a. Introduction (Chapter 1);
b. Quality management, validation and risk management (Chapter 2);
c. Donor evaluation (Chapter 4 for cord blood);
d. Donor testing (Chapter 5);
e. Premises (Chapter 7):
f. Processing (Chapter 8);
g. Storage and release (Chapter 9):
h. Principles of microbiological testing (Chapter 10);
i. Distribution and import/export (Chapter 11);
j. Computerised systems (Chapter 13);
k. Coding, labelling and packaging (Chapter 14);
l. Traceability (Chapter 15);
m. Biovigilance (Chapter 16).

35.2. Serum eye drops

35.2.1. Introduction

Serum eye drops are prepared from the serum component of whole blood or cord blood for use by patients suffering from severe ocular surface diseases, specifically for patients who have either not responded to, or who in their clinician's opinion are unlikely to benefit from, conventional treatments. Serum eye drops can be prepared for autologous use from the patient's own serum or they can be allogeneic, prepared from blood donors or from cord blood. The serum, either undiluted or diluted in physiological saline, is dispensed in small aliquots into dropper bottles or suitable dispensers as eye drops, for application either by the healthcare professional or (for home treatment) by the patient.

Serum eye drops have a potential advantage over traditional therapies for dry eye syndrome and persistent epithelial defects because human serum not only replicates the mechanical functions of tears (lubricating the eyelid, and rinsing particles from the ocular surface), but also serves as a lacrimal substitute, containing many of the same growth factors and other biochemical components that are

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17222 present in natural tears. This is the reason why serum eye drops have become a popular second-line
17223 therapy in dry eye treatment [2-4].

17224 **35.2.2. Donor evaluation**

17225 *35.2.2.1. Autologous setting*

17226 In the case of autologous donation, the risks posed by blood donation must be carefully considered
17227 on an individual basis against the potential benefits from the treatment. Special attention should be paid
17228 to avoiding the development of anaemia, especially where there is repeated collection of blood to prepare
17229 serum eye drops. To overcome problems of co-existing medical conditions related to autologous donors
17230 and delays in treatment because of autologous serum eye drops preparation, the use of allogeneic eye
17231 drops can be considered. Active viral or fungal infection and certain medications that may injure the
17232 cornea are contraindications to donation for serum eye drops.

17233 *35.2.2.2. Allogeneic setting*

17234 Allogeneic serum eye drops can be prepared in advance and be ready for use in emergency cases,
17235 or if patients are not eligible to donate for themselves. Allogeneic serum eye drop donors must meet the
17236 same eligibility criteria as voluntary blood donors [5]. Additional selection criteria over and above these
17237 can be applied according to national requirements. As allogeneic serum eye drops are not lifesaving
17238 products, quarantining of the products for 4 months, followed by a negative nucleic acid test (NAT)
17239 and/or antibody screen on the donors' subsequent donation should be the minimum standard to enhance
17240 product safety. If the initial screening includes NAT, and if appropriate donor-referral criteria and donor-
17241 compliance monitoring are in place to cover the risk of window-period infections, this quarantine period
17242 may not be necessary.

17243 At the end of the quarantine period, the donor should be re-tested for relevant infectious disease
17244 markers, and if the outcome of this further screening is negative, the serum can be released for clinical
17245 application. In addition to general donor selection applicable for allogeneic blood donors, specific
17246 factors such as medications that may change the physiological or immunological state of the eye or that
17247 might injure the cornea should be considered in determining donor suitability for serum eye drops.

17248 *35.2.2.3. Umbilical cord serum*

17249 Umbilical cord blood can be obtained during delivery, and laboratory testing of maternal blood
17250 for infectious diseases is required. Umbilical cord serum contains a higher concentration of growth
17251 factors and neurotropic factors compared with the levels in adult peripheral blood. There is no definitive
17252 evidence demonstrating which components of serum are essential for serum eyedrop efficacy. In
17253 comparison to standard serum, there is limited published evidence available on the use of cord blood
17254 serum in the treatment of various ocular surface diseases that demonstrates efficacy [6, 7].

17255 **35.2.3. Processing and storage**

17256 There must be written protocols for all procedures related to blood and eye drops production. All
17257 measures should be taken to minimise the risk of microbiological contamination, including disinfection
17258 of the phlebotomy site using methods accepted for collection of blood for transfusion. Blood must be
17259 collected in a sterile container/blood bag, without anticoagulant. The collected blood volume depends
17260 on local procedure but cannot be more than for regular blood donors.

17261 The collected blood must be allowed to clot, and the serum must be separated, following validated
17262 protocols used for preparing serum eye drops [8]. The serum can be used undiluted, or diluted with
17263 physiological saline solution to different concentrations. No standard production protocol or optimal
17264 serum concentration has been established to deliver maximal clinical benefit.

17265 Eye-drop preparation must be carried out using aseptic technique. If the process involves open
17266 dispensing, it is required that clean rooms be used for manufacture of eye drops. It is strongly
17267 recommended to use a closed system for aliquoting. Microbiological control for each batch is mandatory
17268 (see Chapter 10). The volume of one aliquot should be adjusted to be no more than one daily dose to

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minimise microbiological growth in the thawed serum during the application period. All bags that are used in the collection, processing and/or aliquots of final packaging must be properly labelled (see Chapter 14).

Eye drops must be stored frozen at <−20 °C and transported in an appropriate container, to maintain the required temperature. Manufacturers must specify the shelf life of serum eye drop products at a defined temperature(s), to the best of their knowledge. This shelf life could be based on studies of the presumptive active components of serum eye drops at the designated storage temperature [9, 10].

The same principles apply to preparing umbilical cord serum eye drops. There must be a written protocol for the preparation of cord blood serum eye drops as described in this section for serum eye drops prepared from whole blood.

Until now no drug substance or mode of action has been specified to explain the beneficial effects of serum eye drops. The risk of bacterial contamination caused by donation and the manufacturing process, as well as during the application period of the thawed product by the patient, should be considered. To improve the risk–benefit ratio, precautions should be taken to avoid bacterial contamination and growth of harmful bacteria by sterile filtration of the final product before freezing the aliquots. However, filtration could also remove some of the presumptive active components of serum eye drops, and this risk should be considered. Other risk-reduction methods, such as a short shelf life, training of the patient and secure product packaging, can be implemented to reduce the bacterial contamination risk during the application period.

35.2.4. Clinical application

The patient must be given appropriate information about the blood collection and testing, and about eye drops preparation. The patient must be provided with written instructions for storage and handling of the eye drops at home, as well as information about the risk of, and signs of, potential bacterial contamination of the product.

35.2.5. Biovigilance/pharmacovigilance

It is strongly recommended that the ophthalmologist monitors the patient's progress in a systematic way to enable collection of data regarding the benefits of using serum eye drops. Any adverse reaction that occurs during usage of eye drops should be notified to the regulatory authority following national regulations.

35.3. Platelet derivatives

35.3.1. Introduction

Platelet-derived products are used in regenerative medicine as source of growth factors and cytokines for the treatment of soft and hard tissue lesions. Each growth factor is involved in a phase of the healing process, such as inflammation, collagen synthesis, tissue granulation and angiogenesis, collectively promoting tissue restitution.

The use of platelet derivatives is an emerging field and its efficacy remains controversial. Several techniques for platelet derivatives are available; however, their applications have been confusing because each method results in a different product with different biology and potential uses. Platelet derivatives have been prepared as platelet-rich plasma (PRP), platelet gel, platelet-rich fibrin (PRF) and platelet lysate eye drops. These products vary in consistency and in composition, for example the concentration of growth factors and cytokines. Depending on the leukocyte and fibrin content, platelet derivatives could be classified into four categories: pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure plaletet-rich fibrin (P-PRF), and leukocyte- and platelet-rich fibrin (L-PRF) [11].

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17313 They are usually used as autologous products and can be prepared at the time of application or in
17314 advance. When they are prepared in advance and stored, this should be done by a blood or tissue
17315 establishment. Allogeneic platelet derivatives can be collected from healthy donors or produced from
17316 umbilical cord blood.

17317 PRP is a concentrated source of autologous platelets, and it contains several different growth
17318 factors and other cytokines, in concentrations 5 to 10 times higher than in standard plasma; PRP can be
17319 used to stimulate healing of soft tissue by injecting this concentrated plasma in the tissue where healing
17320 or effect is desired. There are primarily 3 isomers of platelet-derived growth factor (PDGF), namely αα,
17321 ββ and αβ, 2 transforming growth factors, TGF-β1 and TGF-β2, endothelial growth factor (EGF) and
17322 vascular epidermal growth factor (VEGF). PRP also contains proteins responsible for cell adhesion:
17323 fibrin, fibronectin and vitronectin [12]. The content of bioactive molecules depends on the production
17324 protocol [13]. All the products of this family can be used as liquid solutions or in an activated gel form.
17325 It can therefore be injected, for example in sports medicine, or placed during gelling on a skin wound or
17326 suture.

17327 PRP is used to promote healing of injured tendons, ligaments, muscles and joints, and can be
17328 applied to various musculoskeletal problems. In addition to orthopaedics, other uses include
17329 dermatology, ophthalmology, plastic surgery and dentistry, including oral and maxillofacial surgery. As
17330 of 2017, no large-scale randomised controlled trials have confirmed the efficacy of PRP as a treatment
17331 for musculoskeletal or nerve injuries, the accelerated healing of bone grafts or the reduction of
17332 androgenic hair loss.

17333 The main advantages so far identified in platelet gel derived from umbilical cord blood (CBPG),
17334 as compared with platelet gel from adult platelets, relate to a different profile of growth factor
17335 concentrations, such as a higher content of VEGF and lower content of TGF-β in CBPG. Recent
17336 developments have led to a procedure in which cord blood platelet gel can be prepared, stored in a
17337 cryopreservation bag and applied to the skin ulcer without breaking the sterility chain [14].

17338 Platelet-rich fibrin (PRF) is a second-generation PRP where autologous platelets and leukocytes
17339 form a strong natural fibrin matrix or three-dimensional scaffold. This 'scaffolding' helps localise the
17340 growth factors, essentially increasing their concentration at the desired location to guide tissue
17341 regeneration [15]. PRF has a dense fibrin network with leukocytes, cytokines and structural
17342 glycoproteins, as well as growth factors (e.g. TGF β1, PDGF, VEGF) and glycoproteins, such as
17343 thrombospondin-1. Leukocytes that are concentrated in PRF scaffold play an important role in growth
17344 factor release, immune regulation, anti-infectious activities and matrix remodelling during wound
17345 healing. In addition, due to their elasticity and viscosity, these membranes adhere to the bone surface,
17346 acting as mechanical barriers against the penetration of the epithelium that has faster regeneration
17347 potency than connective tissues [16].

17348 Topical application of a platelet lysate, administered as eye drops, is an alternative therapeutic
17349 option for treatment of ocular surface disorders that do not respond to standard treatment [17]. The
17350 plasma component contains proteins essential for surface lubrication, whereas platelets provide growth
17351 factors (PDGF, EGF and TGF-β) and fibronectin that can promote ocular re-epithelialisation [18]. Eye
17352 drops comprising PRP have been used to treat dry eye syndrome for patients with Sjögren disease, and
17353 ocular chronic graft *versus* host disease (cGvHD) [19], and are used during macular hole surgery. So far,
17354 only studies of small cases series have been published to explore the use of platelet derivatives in
17355 ophthalmology, and further large-scale studies are necessary to demonstrate efficacy.

17356 **35.3.2. Donor evaluation**

17357 In the case of autologous donation, special attention should be paid to the status of coagulation
17358 systems. The use of autologous platelet derivatives avoids the ethical and legal implications of exposing
17359 the patient to the risks (albeit low) of transmission of blood-borne pathogens, although the risk of
17360 infection related to contamination during collection and handling still remains. Disadvantages of

autologous products include a larger individual variability in the quality of platelet derivatives compared with allogeneic products that are prepared from healthy blood through standardised working procedures.

35.3.3. Procurement and processing

Depending on the type of platelet derivatives, they can be prepared from whole blood, from apheresis product, or using other methods of collection, such as small volume bags, tubes or various types of medical devices. Different blood volumes can be used, but the volume of anticoagulant must be proportional to the amount of blood collected. All manipulations during processing carried out in open system must be performed under clean-room conditions.

35.3.3.1. Procurement and processing of platelet-rich plasma

For the preparation of PRP, the blood is drawn with the addition of an anticoagulant, such as citrate dextrose A (ACD-A), to prevent platelet activation prior to its use. The platelets are separated from other blood cells using the two-step centrifugation method. A 30 mL venous blood draw will yield 3-5 mL of PRP, depending on the patient's baseline platelet count, the device used and the technique employed. An initial centrifugation separates red blood cells from PRP, and is followed by a second centrifugation that concentrates platelets in 3-5 mL of the final plasma volume. After the first centrifugation step, the whole blood is separated into three layers: an upper layer that contains mostly platelets and white blood cells, an intermediate thin layer that is known as the buffy coat and is rich in white blood cells, and a bottom layer that consists mostly of erythrocytes.

To produce pure PRP, the upper layer and superficial buffy coat are transferred to an empty sterile tube. The second centrifugation process should be adequate to generate the formation of soft platelet pellets at the bottom of the tube. The upper portion of the volume that is composed mostly of platelet-poor plasma is removed. Platelet pellets are re-suspended in the lower third part of plasma to create the PRP.

Many automated systems for the preparation of PRP which facilitate the preparation of ready-to-apply platelet-rich suspensions in a reproducible manner are commercially available. These systems widely differ in their ability to collect and concentrate platelets, depending on the method and time of its centrifugation. As a result, suspensions of different concentration of platelets and leukocytes are obtained. Differences in the concentrations in platelets and white blood cells influence the diversity of growth factors concentration.

35.3.3.2. Procurement and processing of platelet-rich fibrin

For the preparation of PRF, a sample of blood is collected from the patient in tubes without anticoagulant and the blood is immediately centrifuged. During centrifugation, the platelets are activated when the blood contacts the tube wall.

The duration of time between blood collection and centrifugation is an important factor affecting the success and clinical outcome of this procedure. The majority of PRF preparation protocols recommend immediate (within 2 minutes of collection) centrifugation after blood collection. Delay in centrifugation will result in diffuse polymerisation of fibrin leading to the formation of a small blood clot with irregular consistency. Therefore, a reproducible protocol for PRF production should be followed to obtain a clinically usable fibrin clot with substantial enmeshment of platelets.

After centrifugation, the uppermost of the three layers consists of acellular platelet-poor plasma, the PRF clot is in the middle layer and red blood cells are at the bottom of the tube. After centrifugation, the fibrin clot is removed from the tube and any attached red blood cells are scraped off and discarded.

PRF can also be applied as a membrane; the membrane can be formed in different shapes by squeezing out the fluids present in the fibrin clot using, for example, the stainless steel PRF compression device composed of two spoon-shaped parts [20].

35.3.3.3. Procurement and processing of platelet lysate eye drops

Platelet lysate eye drops are prepared using PRP after freezing–thawing at a final dilution of 30 %. A volume of 40 to 60 mL of peripheral blood anticoagulated with anticoagulant citrate dextrose solution

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A (ACD-A) is collected and centrifuged to obtain an autologous PRP. The platelet concentrate is frozen to −80 °C for at least 60 min and then thawed to induce platelet lysis. The lysate can be diluted with sterile saline solution, and aliquoted into defined doses. A sample for microbiological control must be taken at the time the product was prepared (see Chapter 10). The final product is then frozen again at −20 °C and stored in a freezer. Patients are usually provided with a monthly supply of doses and trained how to thaw the dose, store it for the day at 4°C and safely instil eye drops.

35.3.4. Quality control

The quality of platelet derivatives could be evaluated according to platelet recovery and growth factor contents. Further investigations are required to define standardised protocols for the preparation of high-quality platelet derivatives suitable for different clinical applications, thus making it possible to compare results [21].

35.3.5. Biovigilance

Studies that have evaluated the topical use of platelet derivatives have shown that the application is safe, and no serious adverse events were observed [22, 23]. According to a current literature search on platelet derivatives use, there is no evidence of systemic effects that might limit the use of platelet derivatives, provided that the possible risk of infections is excluded [13]. Few randomised controlled trials have reported adverse events after injection of platelet product; where these occur, they are mostly local side-effects related to venipuncture required for blood collection or (rarely) bad scarring or calcification at the application sites after injection of platelet product.

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17485 # PART D – MONOGRAPHS

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Draft

Monograph 17.1: Ocular tissue for ALK/DALK (Organ cultured cornea)

PRODUCT
Organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
DEFINITION
Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for anterior lamellar keratoplasty.
ESTABLISHED CLINICAL INDICATIONS
• Stromal opacities caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
CRITICAL PROPERTIES
• Stromal transparency. • Viable, functioning endothelium may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) because anterior chamber penetrated. • No evidence of microbial growth.
QUALITY CONTROL REQUIREMENTS
• Stroma is clear and without scars within a 7.50 mm diameter zone. • If applicable, endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2 at end of organ culture storage or ≥ 2 200 cells/mm^2 if only measured before organ culture). • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
STORAGE AND TRANSPORT
• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent in the medium.
SPECIAL LABELLING
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: ➢ Donor age ➢ Storage time in organ culture? ➢ Endothelial cell density, if applicable ➢ Diameter of the central clear zone
SPECIAL WARNINGS
• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the Quality Control Requirements (above).

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Monograph 17.2: Ocular tissue for cold ALK/DALK (Cold stored cornea)

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PRODUCT
Cold-stored corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
DEFINITION
Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for anterior lamellar keratoplasty.
ESTABLISHED CLINICAL INDICATIONS
• Stromal opacities caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
CRITICAL PROPERTIES
• Stromal transparency. • Viable, functioning endothelium may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) because anterior chamber penetrated. • No evidence of microbial growth.
QUALITY CONTROL REQUIREMENTS
• Stroma is clear and without scars within a 7.50 mm diameter zone. • If applicable, endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2). • Not to be used if the organ-culture medium is turbid or becoming yellow.
STORAGE AND TRANSPORT
• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
SPECIAL LABELLING

• In the EU, the grafts must be labelled with the SEC, as applicable.
• Specific information not coded in the SEC must be included in accompanying documentation:
    ➢ Donor age
    ➢ Time in cold-storage medium
    ➢ Endothelial cell density, if applicable
    ➢ Diameter of the clear zone

SPECIAL WARNINGS
• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the Quality Control Requirements (above).

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Monograph 17.3: Ocular tissue for DMEK (Organ cultured cornea)

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PRODUCT
Pre-prepared organ-cultured corneal donor tissue for Descemet membrane endothelial keratoplasty (DMEK)
DEFINITION
Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for DMEK.
ESTABLISHED CLINICAL INDICATIONS
• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
CRITICAL PROPERTIES
• Viable, functioning endothelium. • No evidence of microbial growth.
QUALITY CONTROL REQUIREMENTS
• Descemet membrane is manually peeled off the corneal stroma with the endothelium attached, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy after organ culture but before Descemet membrane peeling (preferably ≥ 2,000 cells/mm^2). • Graft diameter measurement with calliper or trephine (peeled zone ≥ 9.00 mm). • Tears (damage) in Descemet membrane within 9.00 mm zone must be noted in the accompanying documentation. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of the medium are culture-positive for bacteria or fungi.
STORAGE AND TRANSPORT
• The graft is stored in organ-culture medium with or without an osmotically active agent. • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 96 hours.
SPECIAL LABELLING
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information regarding graft placement (free roll or attached to stroma) must be provided. • Specific information not coded in the SEC must be included in accompanying documentation: ➢ Donor age ➢ Storage time in organ culture? ➢ Endothelial cell density ➢ Graft diameter and presence of tears (damage)
SPECIAL WARNINGS
• Not to be used for penetrating keratoplasty.

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Monograph 17.4: Ocular tissue for DMEK (Cold stored cornea)

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PRODUCT
Pre-prepared cold-stored corneal donor tissue for Descemet membrane endothelial keratoplasty (DMEK)
DEFINITION
Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for DMEK.
ESTABLISHED CLINICAL INDICATIONS
• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
CRITICAL PROPERTIES
• Viable, functioning endothelium. • No evidence of microbial growth.
QUALITY CONTROL REQUIREMENTS
• Descemet membrane is manually peeled off the corneal stroma with attached endothelium, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy before Descemet membrane peeling (≥ 2 000 cells/mm^2). • Graft diameter measurement with calliper or trephine (peeled zone ≥ 9.00 mm). • Tears (damage) in Descemet membrane within 9.00 mm zone must be noted in accompanying documentation. • Not to be used if the storage medium is turbid or becoming yellow.
STORAGE AND TRANSPORT
• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
SPECIAL LABELLING

• In the EU, the grafts must be labelled with the SEC, as applicable.
• Specific information regarding graft placement (free roll or attached to stroma) must be provided.
• Specific information not coded in the SEC must be included in accompanying documentation:
  ➢ Donor age
  ➢ Total time in cold storage
  ➢ Endothelial cell density
  ➢ Graft diameter and presence of tears (damage)

SPECIAL WARNINGS
• Not to be used for penetrating keratoplasty.

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Monograph 17.5: Ocular tissue for DSAEK (Organ cultured cornea)
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PRODUCT
Pre-cut organ-cultured corneal donor tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
DEFINITION
Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for DSAEK.
ESTABLISHED CLINICAL INDICATIONS
• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy).
CRITICAL PROPERTIES
• Viable, functioning endothelium. • Stromal transparency. • No evidence of microbial growth. • Regraft for endothelial decompensation.
QUALITY CONTROL REQUIREMENTS
• Endothelial cell density measurement by microscopy after organ culture but before pre-cutting (≥ 2,000 cells/mm²). • Central stromal thickness measurement of the graft (ultrasound or, preferably, optical coherence tomography). • Minimal variation in graft thickness from centre to periphery (≤ 50 % increase in thickness). • Graft diameter measurement with calliper (cap diameter ≥ 9.00 mm). • Replaced anterior corneal cap after pre-cutting. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
STORAGE AND TRANSPORT
• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be kept at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 96 hours depending on the concentration and type of osmotic thinning agent used in the medium.
SPECIAL LABELLING
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: ➢ Donor age ➢ Storage time in organ culture? ➢ Endothelial cell density ➢ Central graft thickness ➢ Cap diameter
SPECIAL WARNINGS
• Not to be used for penetrating keratoplasty.

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Monograph 17.6: Ocular tissue for cold DSAEK (Cold stored cornea)

PRODUCT

Pre-cut cold-stored corneal donor tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

DEFINITION

Human cold-stored corneal donor tissue prepared in a tissue establishment to be used for DSAEK.

ESTABLISHED CLINICAL INDICATIONS

- Primary endothelial failure (mainly Fuchs corneal dystrophy).
- Secondary endothelial failure (mainly pseudophakic bullous keratopathy).
- Regraft for endothelial decompensation.

CRITICAL PROPERTIES

- Viable, functioning endothelium.
- Stromal transparency.
- No evidence of microbial growth.

QUALITY CONTROL REQUIREMENTS

- Endothelial cell density measurement by microscopy before pre-cutting (≥ 2000 cells/mm^2).
- No stromal opacities within a 6.00 mm central zone.
- Central stromal thickness measurement of the graft (ultrasound or, preferably, optical coherence tomography).
- Minimal variation in graft thickness from centre to periphery (≤ 50 % increase in thickness).
- Graft diameter measurement with calliper (cap diameter ≥ 9.00 mm).
- Replaced anterior corneal cap after pre-cutting.
- Not to be used if the storage medium is turbid or becoming yellow.

STORAGE AND TRANSPORT

- The graft is stored in cold-storage medium.
- The graft is refrigerated (2-8 °C).
- The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).

SPECIAL LABELLING

- In the EU, the grafts must be labelled with the SEC, as applicable.
- Specific information not coded in the SEC must be included in accompanying documentation:
    - Donor age
    - Total time in cold storage
    - Endothelial cell density
    - Central graft thickness
    - Cap diameter

SPECIAL WARNINGS

- Not to be used for penetrating keratoplasty.

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Monograph 17.7: Ocular tissue for PK (Organ cultured cornea)
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PRODUCT
Organ-cultured corneal donor tissue for penetrating keratoplasty (PK)
DEFINITION
Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for PK.
ESTABLISHED CLINICAL INDICATIONS
• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, stromal dystrophies, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
CRITICAL PROPERTIES
• Viable, functioning endothelium. • Stromal transparency. • No evidence of microbial growth.
QUALITY CONTROL REQUIREMENTS
• Endothelial cell density measurement by microscopy (≥ 2,000 cells/mm^2 at end of organ-culture storage or ≥ 2,200 cells/mm^2 if only measured before organ culture). • No stromal scars/opacities within a 7.50 mm diameter central zone. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
STORAGE AND TRANSPORT
• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent used in the medium.
SPECIAL LABELLING
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: ➢ Donor age ➢ Storage time in organ culture ➢ Endothelial cell density ➢ Diameter of the clear zone
SPECIAL WARNINGS
• None.

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Monograph 17.8: Ocular tissue for cold PK (Cold stored cornea)
17521

PRODUCT
Cold-stored corneal donor tissue for penetrating keratoplasty (PK)
DEFINITION
Human cold-stored corneal donor tissue prepared in a tissue establishment to be used for PK.
ESTABLISHED CLINICAL INDICATIONS
• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, stromal dystrophies, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
CRITICAL PROPERTIES
• Viable, functioning endothelium. • Stromal transparency. • No evidence of microbial growth.
QUALITY CONTROL REQUIREMENTS
• Endothelial cell density measurement by microscopy before cold storage (≥ 2,000 cells/mm^2). • No stromal scars or opacities within a 7.50 mm diameter central zone. • Not to be used if the storage medium is turbid or becoming yellow.
STORAGE AND TRANSPORT
• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
SPECIAL LABELLING
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: ➢ Donor age ➢ Total time in cold-storage ➢ Endothelial cell density ➢ Diameter of the clear central zone
SPECIAL WARNINGS
• None.

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Draft

Monograph 18.1: Amniotic membrane

TISSUE/CELL PRODUCT

Amniotic membrane (AM) for biological dressing

DEFINITION

Human amniotic membrane obtained from placenta processed in a tissue establishment and preserved for use as biological dressing and substrate for cell growth in different clinical applications.

ESTABLISHED CLINICAL INDICATIONS

- Ophthalmological indications (e.g. ophthalmic corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, chemical or thermal burns).
- Burns (as a temporary or permanent wound dressing).
- Skin lesions of different aetiologies (e.g. vascular ulcers, epidermolysis bullosa, radiation burns).

CRITICAL PROPERTIES

- Preserved structural integrity (barrier function).
- No evidence of microbial growth.
- Adequate graft size.

QUALITY CONTROL REQUIREMENTS

- Microbiological testing (aerobic and anaerobic bacteria, fungi).
- In cases of lyophilisation (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended.

STORAGE AND TRANSPORT

- Lyophilised/freeze-dried AM can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be justified according to the condition range (e.g. ambient temperature, light levels, humidity) that could affect the critical properties of the graft.
- Frozen AM should be stored between –15 and –80 °C and transported at a similar temperature as was used during storage. The shelf life should be justified according to the storage temperature conditions that could impact on the critical properties of the graft.
- Cryoprotected AM grafts are stored at −80 °C (deep frozen) or in liquid or vapour phase of nitrogen at temperatures below −140 °C (cryopreserved). Distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. Transport temperatures of cryoprotected AM above −60 °C must be avoided to ensure the stability of the product and maximum safety for the recipient.
- Glycerolised AM (preserved in glycerol 85 % solution) should be both stored and transported at 2-8 °C.

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, the grafts must be labelled with the SEC, as applicable.
- Specific information not coded in the SEC should be included in accompanying documentation:
  - Key specifications (e.g. graft dimensions in cm^2, appropriate radiation-sensitive labels)
  - Instructions for appropriate rehydration/thawing
  - Information on the composition of antibiotic decontamination cocktail (only in case of intolerance)

SPECIAL WARNINGS

- Dried, *lyophilised, frozen or cryopreserved* allografts should be used within 6 hours after rehydration or thawing (in saline solution).
- Rehydration time of freeze-dried grafts or thawing time of frozen grafts should be defined by originating tissue bank and should be performed under sterile conditions.
- When applicable, rinse out glycerol before use (glycerolised AM).
- After thawing, if applicable, rinse out cryoprotectants before use (cryoprotected AM).
- Do not re-freeze thawed AM grafts.

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Draft

Monograph 19.1: Human-derived acellular dermal matrix (ADM)

TISSUE PRODUCT
Human-derived acellular dermal matrix (ADM)
DEFINITION
Human decellularised dermis from donor skin to be used for wound-healing procedures.
ESTABLISHED CLINICAL INDICATIONS
• **Burn injuries**. As dermal substitute in partial- or full-thickness burns when donor sites are insufficient for autograft or when patient condition is critical, to improve scar quality and to prevent post-burn joint contracture. • **Reconstructive surgery**. As a valid reconstructive tool for any surgical wound in general surgery (e.g. abdominal wall repair), orthopaedic (e.g. rotator cuff reconstruction), oncologic (e.g. breast-conserving surgery), ear nose and throat (ENT) surgery (e.g. myringoplasty, rhinoplasty) and bariatric surgery. • **Ulcers**. Uninfected, chronic ulcers and diabetic foot ulcers, to accelerate the closure and healing rate. • **Full-thickness acute wounds**. As a scaffold to support cell ingrowth and granulation tissue formation and to achieve durable coverage of exposed critical structures (bone, tendons). • **Composite graft technique**. To restore the dermal component by application of human dermis or dermal equivalents, and thin autologous grafts or keratinocytes.
CRITICAL PROPERTIES
• Absence of donor cells (and genetic material). • Graft thickness ranging 0.2-0.8 mm (thin), 0.8-1.2 mm (medium thickness) and 1.3-2.8 mm (thick). • Preserved structural integrity. • Flexibility/pliability. • Resistance to mechanical stress and suturability. • Sterility.
QUALITY CONTROL REQUIREMENTS
• Microbiological testing (aerobic and anaerobic bacteria, fungi). • Graft thickness measurement. • Biocompatibility test (cytotoxicity tests). • Mechanical resistance test. • Morpho-structure evaluation by histological staining (e.g. orcein, Masson, haematoxylin-eosin) or electron microscopy (EM). • Decellularisation process by specific histological staining e.g. DAPI, Hoersch and DNA quantification assay (ng DNA/mg dry tissue). • Residual water test (≤ 5 %) – for lyophilised ADM.
STORAGE AND TRANSPORT
• Depending on the processing methods, most dermal matrices are stored and transported at room temperature (15-25 °C) – e.g. acellular glycerol-preserved, lyophilised and /or irradiated matrices – or refrigerated at 2-8 °C.
SPECIAL LABELLING AND ACCOMPANYING INFORMATION
• In the EU, grafts must be labelled with the SEC, as applicable. • Appropriate radiation-sensitive labels must be used for irradiated ADM. • Thickness and size.
SPECIAL WARNINGS
• Do not sterilise irradiated dermal matrices. • Rehydration of glycerol-preserved or lyophilised allografts is recommended before use (e.g. by washing in saline solution for 20-30 min).

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Draft

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Monograph 19.2: Cryopreserved and deep-frozen skin allografts

TISSUE PRODUCT
Cryopreserved and cryoprotected deep-frozen skin allografts
DEFINITION
Viable skin allografts preserved in a cryoprotective solution.
ESTABLISHED CLINICAL INDICATIONS
• Temporary/semipermanent covering of burns. • Temporary covering of epidermolytic diseases (e.g. toxic epidermolytic necrosis, staphylococcal scalded skin syndrome). • Treatment of hard-to-heal ulcers. • Temporary covering of wounds with exposed bone and/or tendons. • Wound-bed preparation (promoting neovascularisation).
CRITICAL PROPERTIES
• Cell viability: it is maintained by cryopreserved and, to a lesser degree, deep-frozen skin grafts; to consider a graft as viable, a minimum of 20 % of residual cell viability should be achieved. The mean percentage after 10-20 days of storage is reportedly 30-51 % (deep-frozen skin grafts) and 20-60 % (cryopreserved skin grafts) compared to that of the fresh skin, according to different viability assays. • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure). • Graft thickness ranging 0.2-0.8 mm. • Mechanical resistance. • Engraftment to the wound bed.
QUALITY CONTROL REQUIREMENTS
o Microbiological testing (aerobic and anaerobic bacteria, fungi). o Cell viability assessment (if required, depending on the intended application).
STORAGE AND TRANSPORT
• Cryopreserved skin grafts are stored in liquid or in the vapour phase of nitrogen at temperatures lower than −140 °C. • Deep-frozen skin grafts are stored in ultra-low-temperature refrigerators, which generally maintain a temperature lower than −80 °C. • Transport in a nitrogen dry-shipper or in dry ice (according to the processing method and storage temperature).
SPECIAL LABELLING AND ACCOMPANYING INFORMATION
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: ➢ Graft area in cm^2 ➢ Graft thickness ➢ Number of sheets ➢ Decontamination solution composition ➢ Cryoprotective solution composition ➢ Cell viability ➢ Antibodies to *Cytomegalovirus* (CMV) when positive
SPECIAL WARNINGS
• Do not re-freeze thawed skin grafts. • Do not irradiate viable skin graft. • Rinse out cryoprotectants before use.

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Draft

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Monograph 19.3: Glycerol-preserved skin allografts (GPA)

TISSUE PRODUCT

Glycerol-preserved skin allografts (GPA)

DEFINITION

Human split-thickness, glycerol-preserved, de-vitalised skin grafts, with epidermis and upper dermis components for the treatment of skin loss.

ESTABLISHED CLINICAL INDICATIONS

- Temporary biological dressing:
    - in partial-thickness burns,
    - on meshed autografts (sandwich technique),
    - on donor site,
    - after application of *in vitro* cultured keratinocytes.
- Temporary wound coverage after excision in full-thickness burns.
- Temporary coverage in toxic epidermolytic necrolysis.
- Temporary biological dressing for difficult, non-healing wounds, to protect and preserve the viable granulation tissue from desiccation and necrosis (antalgic and antibacterial effect).
- Wound-bed preparation (promoting wound healing).

CRITICAL PROPERTIES

- Graft thickness ranging 0.2-0.8 mm.
- Plain or meshed.
- No evidence of microbial growth.

QUALITY CONTROL REQUIREMENTS

- Intact epidermis and upper dermis (normal morphological structure).
- Microbiological testing (aerobic and anaerobic bacteria, fungi).

STORAGE AND TRANSPORT

- The graft is stored in glycerol 85 % solution to keep the tissue preserved before use.
- The graft is stored at refrigerator temperature (2-8 °C); storage at room temperature (15-25 °C) during transportation is possible.
- Maximum time storage at 2-8 °C for 5 years.

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, the grafts must be labelled with the SEC, as applicable.
- Specific information not coded in the SEC must be included in accompanying documentation or on the label:
    - Size of graft, width and length
    - Graft thickness
    - Plain or meshed

SPECIAL WARNINGS

- Rinse out glycerol before use (incubation in a large volume of sterile 0.9 % NaCl solution for 10 min at room temperature).
- Not to be used if the storage medium is opaque.

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Draft

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Monograph 20.1: Antibiotic decontaminated, cryopreserved femoral artery allograft

TISSUE/CELL PRODUCT

Antibiotic decontaminated, cryopreserved femoral artery allograft

DEFINITION

Human femoral artery. Decontaminated by incubation with one or more antibiotics, and cryopreserved using slow cooling in the presence of a cryoprotectant.

ESTABLISHED CLINICAL INDICATIONS

- Replacement of infected prosthetic vascular allografts.
- Mycotic abdominal aortic aneurysm.
- Chronic ischaemia.
- Critical limb ischemia.

CRITICAL PROPERTIES

- There should be no visible atheroma or calcification present.
- There should be no visible stenosis or dilation present.
- Branching arteries should be 2-3 mm in length.
- There should be no cuts or significant haematomas in the vessel wall.

QUALITY CONTROL REQUIREMENTS

- Post-decontamination, no viable micro-organisms should be detectable on the graft.
- Pre-decontamination, no pathogenic micro-organisms should be detectable on the graft.

STORAGE AND TRANSPORT

- Grafts should be stored at > −140 °C for long term storage. The shelf life at this temperature should be justified by reference to the critical properties.
- Grafts should be transported to the point of use using either liquid nitrogen cooled shippers or solid carbon dioxide refrigeration.
- If grafts are to be stored at −80 °C (or other temperature > −140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

SPECIAL LABELLING

- The grafts should be marked with the SEC.
- Specific information not coded in the SEC must be included in accompanying documentation:
    - Donor age and gender
    - Key dimensions (proximal and distal annular diameter and length)
    - Identity of any residual processing chemicals (antibiotics and cryoprotectants)
    - Instructions for appropriate thawing

SPECIAL WARNINGS

- Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale.
- Must not be refrozen once thawed.

Draft

Monograph 20.2: Antibiotic decontaminated, cryopreserved heart valve allograft

TISSUE/CELL PRODUCT

Antibiotic decontaminated, cryopreserved heart valve allograft

DEFINITION

Human heart valve, including the base of the aorta/pulmonary trunk and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled cooling in the presence of a cryoprotectant.

ESTABLISHED CLINICAL INDICATIONS

- Tetralogy of Fallot.
- Double output right ventricle.
- Truncus arteriosus.
- Transposition of the great vessels.
- Ventricular septal defect.
- Pulmonary stenosis.
- Pulmonary atresia.
- Aortic stenosis.
- Aortic insufficiency.
- Absent pulmonary valve syndrome.
- Endocarditis.
- Ross procedure.

CRITICAL PROPERTIES

- There should be no visible calcification present in the valve or associated vessel.
- Pulmonary valves: there must be a rim of myocardium of at least 2 mm depth surrounding the base of the vessel.
- Aortic valves: there must be a rim of myocardium or mitral leaflet of at least 2 mm depth surrounding the base of the vessel.
- The associated pulmonary artery should not be cut below the level of the valve leaflet cusps.
- The native biomechanical and hydrodynamic properties of the valve should not be altered by the decontamination and preservation protocols applied, as demonstrated by a functionality test.

QUALITY CONTROL REQUIREMENTS

- Post-decontamination, no viable micro-organisms should be detectable on the graft.
- Pre-decontamination, no pathogenic micro-organisms should be detectable on the graft.
- Functionality tests (such as competency).

STORAGE AND TRANSPORT

- Grafts should be stored at < −140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties.
- Grafts should be transported to the point of use using either liquid nitrogen cooled shippers or solid carbon dioxide refrigeration.
- If grafts are to be stored at −80 °C (or other temperature > −140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
- If graft is shipped in dry ice, it should not be returned to storage at < −140 °C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

SPECIAL LABELLING

- The grafts should be marked with the SEC.
- Specific information not coded in the SEC must be included in accompanying documentation:
  - Donor age and gender
  - Key dimensions (annular diameter and length/diameter of associated vessels)
  - Identity of any residual processing chemicals (antibiotics and cryoprotectants)
  - Instructions for appropriate thawing

SPECIAL WARNINGS

- Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale.
- Must not be refrozen once thawed.

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Draft

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Monograph 21.1: Cancellous bone chips
17540

TISSUE/CELL PRODUCT
Allogeneic cancellous bone chips
DEFINITION
Cancellous bone, also referred to as trabecular bone or spongy bone, is the porous non-mineralised component of bone that has a honeycomb or sponge-like appearance. The bone matrix is organised into a three-dimensional lattice of bony processes (trabeculae) arranged along lines of stress. Cancellous bone chips are sawn or ground to various sizes (generally 1-10 mm diameter/edge length). Cancellous chips provide an osteoconductive matrix for autologous bone remodelling and healing.
ESTABLISHED CLINICAL INDICATIONS
• Cancellous chips fill bony defects in almost all parts of the skeletal system, including periodontal regions.
CRITICAL PROPERTIES
• Cancellous chips should only be prepared from bone tissue with suitable morphology and density (contraindications: osteoporosis). The essential native biomechanical properties of the bone must not be significantly altered by the processing protocols. • Processing steps should largely reduce fat and remove blood cells and bone marrow. • Cancellous chips should undergo a process that guarantees absence of any pathogens and viral inactivation. • The bone must not be rendered cytotoxic by the processing protocol.
QUALITY CONTROL REQUIREMENTS
• Microbiological testing (aerobic and anaerobic bacterial, fungi). • In case of lyophilisation (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended.
STORAGE AND TRANSPORT
• Lyophilised/freeze-dried cancellous chips can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided. • Frozen cancellous chips should be stored between –15 and –80 °C and transported at or below –15 °C. • The shelf-life should be justified according to the condition range (e.g. ambient temperature, light levels, humidity) that could impact on the critical properties of the graft.
SPECIAL LABELLING AND ACCOMPANYING INFORMATION
• In the EU, the grafts must be labelled with the SEC, as applicable. • Specific information not coded in the SEC should be included in accompanying documentation: ➢ Key specifications (e.g. graft dimensions, weight of pack) ➢ Instructions for appropriate rehydration/thawing ➢ Information about potential risks (e.g. transmission of infectious diseases)
SPECIAL WARNINGS
• Cancellous chips should be used within 6 hours after rehydration or thawing. • Rehydration time of freeze-dried grafts or thawing time of frozen grafts must be defined by originating tissue establishment and must be performed under sterile conditions. • In case of lyophilisation (freeze drying): residual moisture or available water.

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Draft

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Monograph 21.2: Cortical bone struts
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TISSUE/CELL PRODUCT

Allogeneic cortical bone struts

DEFINITION

Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons). Cortical strut allografts are diaphyseal segments of bone allograft. Cortical struts are made from full circumferential segments, hemicylinders or flat-planed struts from femur, tibia, humerus or full circumferential segments of fibula by sawing into several sizes. Cortical strut allografts unite to host bone through callus formation, restoring bone stock, and can be used as an onlay biological plate.

ESTABLISHED CLINICAL INDICATIONS

- Revision arthroplasty and periprosthetic fractures.
- Bridging of structural defects in long bones.
- Buttress in limb-salvage procedures.

CRITICAL PROPERTIES

- Cortical struts should only be prepared from bone tissue with suitable morphology and density (contraindications: osteoporosis, osteomalacia).
- Processing steps should largely reduce fat and remove blood and bone marrow. The bone must not be rendered cytotoxic by the processing protocol.
- Cortical struts should undergo a process that guarantees absence of any pathogens and viral inactivation. In cases of irradiation, the effect on biomechanical properties of cortical bone has to be considered.

QUALITY CONTROL REQUIREMENTS

- Microbiological testing (aerobic and anaerobic baterical, fungi).
- In case of lyophilisation (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended.

STORAGE AND TRANSPORT

- Lyophilised/freeze-dried cortical struts can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided.
- Frozen cortical struts should be stored between –15 and –80 °C and transported ≤ –15 °C.
- The shelf-life should be justified according to the condition range (e.g. ambient temperature, light levels, humidity) that could impact on the critical properties of the graft.

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, the grafts must be labelled with the SEC, as applicable.
- Specific information not coded in the SEC should be included in accompanying documentation:
  - Key specifications (e.g. graft dimensions, weight of pack)
  - Instructions for appropriate rehydration/thawing
  - Information about potential risks (e.g. transmission of infectious diseases)

SPECIAL WARNINGS

- Cortical struts should be used within 6 hours after rehydration or thawing.
- Rehydration time of freeze-dried grafts or thawing time of frozen grafts must be defined by originating tissue establishment and must be performed under sterile conditions.
- In case of lyophilisation (freeze drying): residual moisture or available water.

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Draft

Monograph 21.3: Patellar tendon allografts

TISSUE/CELL PRODUCT

Patellar tendon allografts (bone–tendon–bone ligaments)

DEFINITION

The patellar ligament is the distal portion of the common tendon of the *M. quadriceps femoris*, which continues from the patella to the tibial tuberosity. It is also called the patellar tendon as it is a continuation of the quadriceps tendon.

ESTABLISHED CLINICAL INDICATIONS

- Reconstruction of the anterior cruciate ligament (ACL).
- Extensor mechanism injuries in the knee joint.

CRITICAL PROPERTIES

- Patellar tendon allografts are procured as a bone-tendon-bone unit, with or without a part of the quadriceps tendon (length 10-20 cm, width 10-40 mm, space between bone blocks 30–80 mm) with a block from the tibial tuberosity (approximately 3.5 cm long and at least 2 cm wide). Tendons 2 cm wide or more are suitable for splitting to provide two patellar tendon allografts.
- Patellar tendon allografts should undergo a process that guarantees absence of any pathogens and viral inactivation. These techniques may have a detrimental effect on both the biomechanical and biological properties of the graft and this effect must be considered.
- Processing steps should largely reduce fat and remove blood and bone marrow. The bone must not be rendered cytotoxic by the processing protocol.

QUALITY CONTROL REQUIREMENTS

- Microbiological testing.
- In case of lyophilisation (freeze drying) a residual moisture of 5-15 % (w/w) is recommended.

STORAGE AND TRANSPORT (IF CRITICAL – JUST THE TEMP AND THE LENGTH OF TIME)

- Lyophilised/freeze-dried patellar tendon allografts can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided.
- Frozen patellar tendon allografts should be stored between –15 and –80 °C and transported at or below –15 °C.
- *The shelf-life should be justified according to the condition range (e.g. ambient temperature, light levels, humidity) that could impact on the critical properties of the graft.*

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, the grafts must be labelled with the SEC, as applicable.
- Specific information not coded in the SEC should be included in accompanying documentation:
  - Key specifications (e.g. graft dimensions, weight of pack)
  - Instructions for appropriate rehydration/thawing
  - Information about potential risks (e.g. transmission of infectious diseases)

SPECIAL WARNINGS

- Patellar tendon allografts should be used within 6 hours after rehydration or thawing.
- Rehydration time of freeze-dried grafts or thawing time of frozen grafts must be defined by originating tissue establishment and must be performed under sterile conditions.
- In case of lyophilisation (freeze drying): residual moisture or available water.

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Monograph 22.1: Haematopoietic progenitor cells from bone marrow – HPC(M)

TISSUE/CELL PRODUCT

Haematopoietic progenitor cells (HPC) from the bone marrow (M)– HPC(M)

DEFINITION

HPCs are found in small numbers in bone marrow. The infused HPC(M) can originate from the recipient (autologous) or from another individual (allogeneic). They can be used as fresh unmanipulated product or can be further processed (e.g. buffy-coat preparation, cell selection, cryopreservation).

ESTABLISHED CLINICAL INDICATIONS

- Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation).
- Establishment of donor chimerism (allogeneic transplantation).

CRITICAL PROPERTIES

- Cellularity/viability
  for *autologous transplantation*:
    - Nucleated cell dose: >1.0-2.0 × 10^8/kg recipient body weight,
    - Viable CD34$^+$ cell dose: ≥2.0 × 10^6/kg recipient body weight;

  for *allogeneic transplantation*:
    - Nucleated cell dose: ≥2.0-3.5 × 10^8/kg recipient body weight,
    - Viable CD34$^+$ cell dose: ≥2.0-3.50 × 10^6/kg recipient body weight.
- Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient).
- In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient body weight.
- In cases of cryopreserved HPC(M), DMSO volume should be less than 1 mL/kg recipient body weight.

QUALITY CONTROL REQUIREMENTS

- Nucleated cell count.
- Enumeration of viable CD34$^+$ cells.
- Microbiological testing.
- ABO Rh blood group for allogeneic products.
- Measurement of residual ABO-incompatible red cell volume.

STORAGE AND TRANSPORT

- Fresh HPC(M) are stored and transported at room temperature (15-25 °C) or refrigerated (2-8 °C).
- Fresh HPC(M) can be stored up to 72 hours without cryopreservation.
- Cryopreserved HPC(M) are stored and transported at temperatures equal or below −140 °C.
- Cryopreserved HPC(M) can be stored for up to 10 years or longer.

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, grafts must be labelled with the SEC as applicable.
- If applicable: warning statements and /or biohazard label.
- Specific information not coded in the SEC must be included in accompanying documentation:
    - Donor name (autologous or related donors) or donor ID (unrelated donors)
    - Recipient name (if permitted), recipient ID (if applicable)
    - Nucleated cell count and viable CD34$^+$ cell enumeration
    - ABO Rh blood group
    - Volume
    - Identity of the collection facility and/or donor registry
    - Identity of processing and distribution facility
    - Instructions for appropriate thawing, if applicable

SPECIAL WARNINGS (IF NEEDED)

- Do not irradiate.
- Properly identify intended recipient and product.

Draft

- For use by intended recipient only.
- For autologous use only, if applicable.
- Do not use leukoreduction filters.
- Use immediately after thawing.
- If presence of microbial contamination, consider antibiotic treatment in the recipient.

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Monograph 22.2: Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)

TISSUE/CELL PRODUCT

Haematopoietic progenitor cells (HPCs) from umbilical cord blood (CB)– HPC(CB)

DEFINITION

HPCs are found in umbilical cord blood (CB). The infused HPC(CB) can originate from the recipient (autologous) or from another individual (allogeneic). CB units are distributed cryopreserved as whole blood or buffy-coat enriched.

ESTABLISHED CLINICAL INDICATIONS

- Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation).
- Establishment of donor chimerism (allogeneic transplantation).

CRITICAL PROPERTIES

- Cellularity/viability
  For autologous transplantation:
    - Nucleated cell dose: ≥2.0 × 10^7/kg body weight (after thawing),
    - $CD34^+$ cell dose: ≥1.2 × 10^5/kg body weight (after thawing);

  For allogeneic transplantation:

  CB units 6/6 or 5/6 HLA-matched
    - Nucleated cell dose: >2.0 × 10^7/kg body weight (after thawing),
    - $CD34^+$ cell dose: >1.2 × 10^5/kg body weight (after thawing);

  CB units 4/6 HLA-matched
    - Nucleated cell dose: >3.0 × 10^7/kg body weight (after thawing),
    - $CD34^+$ cell dose: >1.7 × 10^5/kg body weight (after thawing).
- Absence of microbial contamination (the presence of microbial contamination will not preclude release but may indicate the need for antibiotic treatment in the recipient).
- In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient body weight.

QUALITY CONTROL REQUIREMENTS

- Total nucleated cell count.
- Viable $CD34^+$ cell enumeration.
- Viability of $CD45^+$ and $CD34^+$ cells.
- Microbiological testing.
- ABO Rh blood group and confirmatory HLA typing.
- Measurement of residual ABO incompatible red cell volume.
- CFU or other validated potency assay.

STORAGE AND TRANSPORT

- Fresh HPC(CB) are stored and transported at room temperature (15-25 °C) or refrigerated (2-8 °C).
- Fresh HPC(CB) can be stored up to 72 hours without cryopreservation.
- Cryopreserved HPC(CB) are stored and transported at temperatures equal or below −150 °C.
- Cryopreserved HPC(CB) can be stored for more than 10 years.

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, grafts must be labelled with the SEC as applicable.
- If applicable: warning statements and /or biohazard label.
- Specific information not coded in the SEC must be included in accompanying documentation:
    - Donor name (autologous or related donors) or donor ID (unrelated donors)
    - Recipient name, recipient ID (if applicable)
    - Nucleated cell count and viable $CD34^+$ cell enumeration
    - Result of a potency assay
    - ABO Rh blood group
    - Volume

Draft

➢ Identity of the collection facility and /or donor registry ➢ Identity of processing and distribution facility ➢ Instructions for appropriate thawing and washing if units have not been red cell reduced prior to cryopreservation ➢ Circular of Information brochure (including: handling instructions for the use of cellular therapy products, with indications, contraindications, side-effects and hazards, dosage and infusion recommendations)
SPECIAL WARNINGS (IF NEEDED)
• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing.

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Monograph 22.3: Haematopoietic progenitor cells from peripheral blood (apheresis A) - HPC(A)

PRODUCT
Haematopoietic progenitor cells (HPCs) from the peripheral blood (apheresis A) - HPC(A)
DEFINITION
HPC(A) are procured by apheresis from the mononuclear cell fraction of circulating blood after their mobilisation from the bone marrow. The infused HPC(A) can originate from the recipient (autologous) or from another individual (allogeneic). They can be used as fresh unmanipulated product or further processed (e.g. cell selection, cryopreservation).
ESTABLISHED CLINICAL INDICATIONS
• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
CRITICAL PROPERTIES
• Cellularity/viability for *autologous transplantation*: ➢ Viable CD34$^+$ cell dose: ≥2.0 × 10^6/kg recipient body weight; for *allogeneic transplantation:* ➢ Target viable CD34$^+$ cell dose: approximately >5.0 × 10^6/kg recipient body weight, ➢ Minimum viable CD34$^+$ cell dose: 1.5-3.5 × 10^6/kg body weight. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient weight. • In case of cryopreserved HPC(A), DMSO volume should be less than 1 mL/kg recipient body weight.
QUALITY CONTROL REQUIREMENTS
• Nucleated cell count. • Viable CD34$^+$ cell enumeration. • Microbiological testing. • ABO Rh blood group for allogeneic products. • Measurement of residual ABO-incompatible red cell volume.
STORAGE AND TRANSPORT
• Fresh HPC(A) are stored and transported at room temperature (15-25 °C) or refrigerated (2-8 °C). • Fresh HPC(A) can be stored up to 72 hours without cryopreservation. • Cryopreserved HPC(A) are stored and transported at temperatures equal or below −140 °C. • Cryopreserved HPC(A) can be stored for up to 10 years or longer.
SPECIAL LABELLING AND ACCOMPANYING INFORMATION
• In the EU, grafts must be labelled with the SEC as applicable. • If applicable: warning statements and /or biohazard label. • Specific information not coded in the SEC must be included in the accompanying documentation: ➢ Donor name (autologous or related donors) or donor ID (unrelated donors) ➢ Recipient name (if permitted), recipient ID (if applicable) ➢ Viable CD34$^+$ cell enumeration ➢ ABO Rh blood group ➢ Volume ➢ Identity of the collection facility and /or donor registry ➢ Identity of processing and distribution facility ➢ Instructions for appropriate thawing (if applicable)
SPECIAL WARNINGS (IF NEEDED)
• Do not irradiate. • Properly identify intended recipient and product.

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- For use by intended recipient only.
- For autologous use only, if applicable.
- Do not use leukoreduction filters.
- Use immediately after thawing.
- If presence of microbial contamination, consider antibiotic treatment in the recipient.

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Monograph 22.4: Mononuclear cells from unstimulated peripheral blood (apheresis A) – MNC(A)

TISSUE/CELL PRODUCT

Mononuclear cells (MNC) from unstimulated peripheral blood (apheresis A) – MNC(A)

DEFINITION

Unstimulated mononuclear cells are procured by apheresis from the circulating blood. The procured cells can originate from the recipient (autologous) or from another individual (allogeneic). Unstimulated mononuclear cells can be used as fresh non-manipulated products or further processed (e.g. cryopreservation, cell selection, starting material for ATMPs).

ESTABLISHED CLINICAL INDICATIONS

- MNC(A) after allogeneic stem cell transplantation from the original HPC donor are used in cases of relapse and mixed chimerism or as relapse prophylaxis to enhance the graft-*versus*-malignancy effect, to promote immune reconstitution and prevent infection complications.
- MNC(A) for generation of cellular therapies and ATMPs (e.g. NK-cell therapy, virus-specific T-cells, CAR-T cells).

CRITICAL PROPERTIES

- Cellularity/viability
  After allogeneic transplantation to enhance immunity and graft-*versus*-malignancy effect:
  - Escalating cell doses of $CD3^+$ cells, depending on the clinical situation and the transplant setting (e.g. in case of relapse from 1.0×10^6/kg to 1.0×10^8/kg body weight),
  - $CD3^+$ cell dose $>1.0 \times 10^8$/kg body weight per infusion should be avoided due to increased risk of graft-*versus*-host disease;

  As starting material for generation of cellular therapy and ATMPs:
  - Required cell dose according to the specific protocol.
- Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient).

QUALITY CONTROL REQUIREMENTS

- Nucleated and mononuclear cell count.
- Viability.
- Viable $CD3^+$ cells enumeration.
- Microbiological testing.
- ABO Rh blood group for allogeneic products.

STORAGE AND TRANSPORT

- Fresh MNC(A) are stored and transported at room temperature (15-25 °C) or refrigerated (2-8 °C).
- Fresh MNC(A) can be stored up to 72 hours without cryopreservation.
- Cryopreserved MNC(A) are stored and transported at temperatures equal or below −140 °C.
- Cryopreserved MNC(A) can be stored for up to 10 years or longer.

SPECIAL LABELLING AND ACCOMPANYING INFORMATION

- In the EU, grafts must be labelled with the SEC as applicable.
- If applicable: warning statements and /or biohazard label.
- Specific information not coded in the SEC must be included in the accompanying documentation:
  - Donor name (autologous or related donors) or donor ID (unrelated donors)
  - Recipient name, recipient ID (if applicable)
  - Total nucleated and mononuclear cell count
  - Viable $CD3^+$ cell count
  - ABO Rh blood group (allogeneic products)
  - Volume
  - Identity of the collection facility and /or donor registry
  - Identity of processing and distribution facility
  - Instructions for appropriate thawing, if applicable

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SPECIAL WARNINGS (IF NEEDED)
• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing (if applicable). • If presence of microbial contamination, consider antibiotic treatment in the recipient.

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17569 # Monograph 27.1: Vitrified oocytes for non-partner donation
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TISSUE/CELL PRODUCT
Vitrified oocytes for non-partner donation
DEFINITION
Vitrified human oocytes obtained after controlled ovarian hyperstimulation, to be used for non-partner oocyte donation in *in vitro* oocyte fertilisation. Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised.
ESTABLISHED CLINICAL INDICATIONS
Women needing a non-partner oocyte donation to achieve a pregnancy. The most common indications are: • Premature ovarian failure, either primary or secondary, including surgical oophorectomy, irreversible gonadal damage after chemotherapy or radiotherapy, Turner syndrome and other chromosomal disorders causing gonadal dysgenesis. • Start of natural menopause, or other age-related infertility. • Carriers of genetic diseases that cannot be treated by PGT-M. • Carriers of structural abnormalities that cannot be treated by PGT-SR. • Carriers of mitochondrial diseases.
CRITICAL PROPERTIES
• Maturation status of the oocytes is metaphase II (MII). • Absence of giant oocyte size.
QUALITY CONTROL REQUIREMENTS
• Oocytes vitrified 38–40 h post ovulation induction when possible. • Morphological assessment of oocyte size and maturation status.
STORAGE AND TRANSPORT
• Temperature below −140 °C.
SPECIAL LABELLING
• In the EU, oocyte samples originating from non-partner donation and transported to another tissue establishment for human application must be labelled with a full SEC code. • Specific information not coded in the SEC must be included in accompanying documentation, for example: ➢ Number of shipped samples ➢ Type of medium used for storage, including batch information ➢ Type of storage device ➢ Number of oocytes per storage device ➢ Instructions for warming
SPECIAL WARNINGS
• Any registered events and reactions in the donor file that may have implications for usage.

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Monograph 27.2: Cryopreserved sperm for non-partner donation
17575

TISSUE/CELL PRODUCT
Cryopreserved sperm for non-partner donation
DEFINITION
Cryopreserved human spermatozoa obtained by ejaculation, to be used in non-partner sperm donation for intra-uterine insemination or *in vitro* oocyte fertilisation. Processed by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled cooling rate down to a temperature of approximately −100 °C and thereafter transferred to liquid nitrogen.
ESTABLISHED CLINICAL INDICATIONS
Couples or individuals in need of a non-partner sperm donation to achieve a pregnancy, either by intrauterine insemination or by *in vitro* fertilisation of oocytes. The most common indications are: • Azoospermia. • Fertilisation failure. • Absence of a male partner. • Carriers of genetic diseases that cannot be treated by PGT-M. • Carriers of structural abnormalities that cannot be treated by PGT-SR.
CRITICAL PROPERTIES
• Presence of post-thaw viable and motile sperm.
QUALITY CONTROL REQUIREMENTS
• The number of motile spermatozoa after test thawing must be adequate for the intended use: IUI, routine IVF or microinjection (ICSI).
STORAGE AND TRANSPORT
• Cryopreserved sperm can be shipped in liquid nitrogen or on carbon dioxide ice.
SPECIAL LABELLING
• In the EU, sperm samples originated from non-partner donation and transported to another tissue establishment for human application must be labelled with a full SEC code. • Specific information not coded in the SEC must be included in accompanying documentation, for example: ➢ Number of shipped samples ➢ Pre-cryopreservation sperm concentration and motility ➢ Number of motile spermatozoa per device ➢ Type of medium used for storage, including batch information ➢ Type of storage device ➢ Instructions for thawing • In cases when the container is too small to include the DIS on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
SPECIAL WARNINGS
• Any registered event and reactions in the donor file that may have implications for usage.

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PART E – GOOD PRACTICE GUIDELINES FOR TISSUE ESTABLISHMENT

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Introduction

High-quality, safe and efficacious procedures in relation to the donation, procurement, importation, testing, processing, preservation and storage of human tissues and cells for human application are essential for donors and recipients alike. As health products of an exceptional nature, all Member States should endeavour to promote high level of safety and quality of these substances when used for human application to safeguard public health. This objective should be attained, maintained and continually optimised through the identification and implementation of key quality and safety criteria in relation to donation, procurement, importation, testing, processing, preservation, storage and distribution in Tissue Establishments

In the field of blood and blood components, Good Practice Guidelines are included in the "Guide for the preparation, use and quality assurance of blood components" since the 18th Edition, published in 2015. In 2016, Directive 2005/62/EC was amended to require EU Member States to take into account the Good Practice Guidelines jointly developed by the Commission and the European Directorate for the Quality of Medicines and Healthcare of the Council of Europe and published by the Council of Europe [1.]

Following this approach, and with the aim to promote and assure a high level of quality in the field of human tissues and cells , the European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM/CoE) took the decision to incorporate in the 4th edition of the Guide to the Quality and Safety of Tissues and Cells for Human Application equivalent Good Practices Guidelines (GPG) for this field .

These guidelines do not introduce new requirements but rather consolidate that guidance which is already defined in existing legislation and scientific guidelines. The GPG are therefore intended to elaborate on the basic requirements set out in the European Union Tissues and Cells Directives (EUTCD) and to detail the key elements which should be defined and controlled within the quality system of tissue establishments that are required to comply with EUTCD.

The GPG incorporate and elaborate on the associated recommendations from the main chapters of the Guide to the quality and safety of tissues and cells for human application, relevant elements derived from the detailed principles of GMP (as referred to in Article 47 of EU Directive 2001/83/EC), the results of relevant EU funded projects and expert opinion consistent with current scientific knowledge.

The GPG should be seen as a complementary document for tissue establishments and tissue establishment inspectors or auditors that describes in detail, and from a practical point of view, the key elements for achieving comprehensive quality management in a tissue establishment.

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1 Directive 2016/1214 amending Directive 2005/62/EC

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17632 # GOOD PRACTICE GUIDELINES

17633 for tissues establishments that follow EU directives

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17635 ## 1. General principles

17636 ### 1.1. General principles

17637 1.1.1. The term “tissue establishment” (TE) became widely used in Europe following
17638 publication of the EU Tissues and Cells Directive 2004/23/EC, which defines it as: “*a*
17639 *tissue bank or a unit of a hospital or another body where activities of processing,*
17640 *preservation, storage or distribution of human tissues and cells for human application*
17641 *are undertaken. It may also be responsible for procurement or testing of tissues and*
17642 *cells”.*

17643 1.1.2. In the field of Medically Assisted Reproduction (MAR), the term “tissue establishment”
17644 refers to the laboratories in MAR centres or clinics as well as banks of gametes. These
17645 centres or clinics often include clinical units in which the patients are treated. In the
17646 context of these guidelines, the term “tissue establishment” will be used and refer to
17647 all the banks, units, centres and clinics mentioned above.

17648 ### 1.2. EU tissues and cell legislation

17649 1.2.1. The EU tissues and cells directives created a benchmark for the standards that must
17650 be met if carrying out any activity involving tissues and cells for human application,
17651 including gametes, embryos and germinal tissue. The directives also require that
17652 systems be put in place to ensure that all the tissues and cells used in human
17653 applications are traceable from donors to recipients and vice versa.

17654 1.2.2. Directive 2004/23/EC of the European Parliament and of the Councill of 31 March 2004
17655 applies to the donation, procurement, testing, preservation, storage and distribution
17656 of human tissues and cells intended for human application (including reproductive
17657 cells used in MAR procedures). It introduced obligations on EU member states,
17658 authorities, from supervision of human tissue and cell procurement and authorising
17659 and inspecting tissue establishment, to ensuring traceability and vigilance as well as
17660 maintaining a publicly accessible register of national tissues establishments. This
17661 Directive also laid down the rules on donor selection and evaluation and the quality
17662 and safety of tissues and cells (e.g. quality management, tissue and cells reception,
17663 processing and storage conditions).

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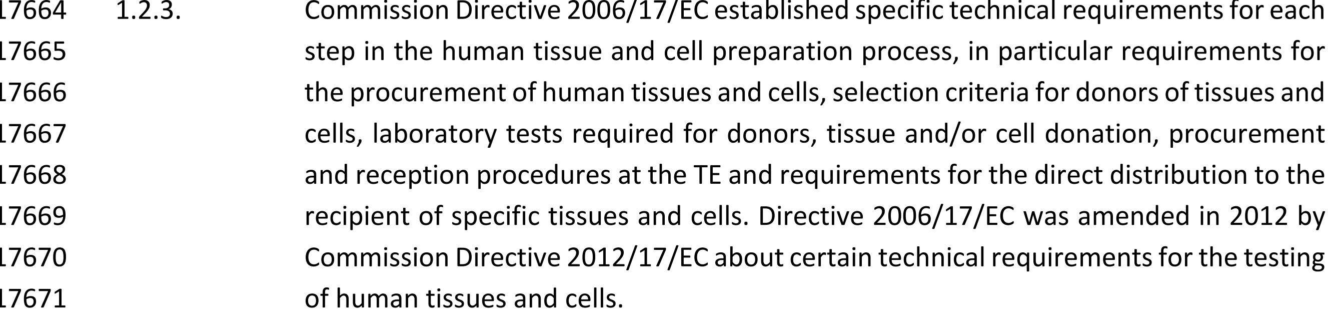

1.2.3. Commission Directive 2006/17/EC established specific technical requirements for each step in the human tissue and cell preparation process, in particular requirements for the procurement of human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for donors, tissue and/or cell donation, procurement and reception procedures at the TE and requirements for the direct distribution to the recipient of specific tissues and cells. Directive 2006/17/EC was amended in 2012 by Commission Directive 2012/17/EC about certain technical requirements for the testing of human tissues and cells.

1.2.4. Commission Directive 2006/86/EC includes traceability requirements, notification of serious adverse reactions and events (SAREs) as well as certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

1.2.5. In 2015, two new Commission Directives were adopted, one an implementing directive on the procedures for verifying equivalent standards of quality and safety of imported tissues and cells (Directive 2015/566). The second one amending Directive 2006/86/EC providing detailed requirements on the coding of human tissues and cells (Directive 2015/ 565).

1.3. Using this guide

1.3.1. These guidelines are based on a quality management system (QMS) approach. They form the basis of good practice in all TE and should be used in preparation for both inspection and continuous improvement.

1.4. For each topic, the guidelines aim to provide sufficient detail for establishments to be made aware of the essential matters which should be considered at least in the context of a risk-based analysis which takes full account of the specific protocols and risk mitigation strategies of each establishment relevant to the risks associated with the processing, testing and implantation of the types of tissues and cells concerned.

1.5. Development of the Good Practice Guidelines

High-quality, safe and efficacious procedures in relation to the donation, procurement, importation, testing, processing, preservation and storage of human tissues and cells for human application are essential for donors and recipients alike. As health products of an exceptional nature, all Member States should endeavour to promote a high level of safety and quality of these substances when used for human application to safeguard public health. This objective should be attained, maintained and continually optimised through the identification and implementation of key quality and safety criteria in relation to donation, procurement, importation, testing, processing, preservation, storage and distribution.

These guidelines do not introduce new requirements but rather consolidate that guidance which is already defined in existing legislation and guidelines. The guidelines

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17702 are therefore intended to elaborate on the basic requirements set out in the European
17703 Union Tissues and Cells Directives (EUTCD) and to detail the key elements which should
17704 be defined and controlled within the quality system of tissue establishments that are
17705 required to comply with EUTCD. The guidelines incorporate and elaborate on the
17706 associated recommendations from the main chapters of the Guide to the quality and
17707 safety of tissues and cells for human application, relevant elements derived from the
17708 detailed principles of GMP (as referred to in Article 47 of EU Directive 2001/83/EC),
17709 the results of relevant EU funded projects and expert opinion consistent with current
17710 scientific knowledge

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17712
2. Quality Management System (QMS)

17713
2.1. *General requirements*

17714 2.1.1. Quality Management is a wide-ranging concept covering all matters, which individually
17715 or collectively influence the quality of tissues and cells. It is the sum total of the
17716 organised arrangements made with the objective of ensuring that tissues are of the
17717 quality required for their intended use. Quality Management therefore incorporates
17718 Good Practice.

17719 2.1.2. Each tissue establishment must develop and maintain a Quality System which
17720 facilitates meeting all the relevant minimum requirements identified in the EU tissues
17721 and cells directives and which is based on the principles of good practice, incorporating
17722 quality risk management and taking into account the relevant elements of EU Good
17723 Manufacturing Practices (GMP) Directive 2001/83/EC.

17724 2.1.3. Quality must be recognised as being the responsibility of all persons involved in the
17725 processes of the tissue establishment, with management ensuring a systematic
17726 approach towards quality and the implementation and maintenance of a Quality
17727 System.

17728 2.1.4. Attainment of this quality objective is the responsibility of senior management. It
17729 requires the participation and commitment both of staff in many different
17730 departments and at all levels within the organisation and of the organisation's
17731 suppliers and distributors. To achieve this quality objective reliably there must be a
17732 comprehensively designed and correctly implemented Quality System incorporating
17733 Good Practice and Quality Risk Management.

17734 2.1.5. Each actor in the supply chain should establish, document, and fully implement a
17735 comprehensively designed Quality System to deliver Quality Assurance based on the
17736 principles of Quality Risk Management by incorporating Good Practice and Quality
17737 Control.

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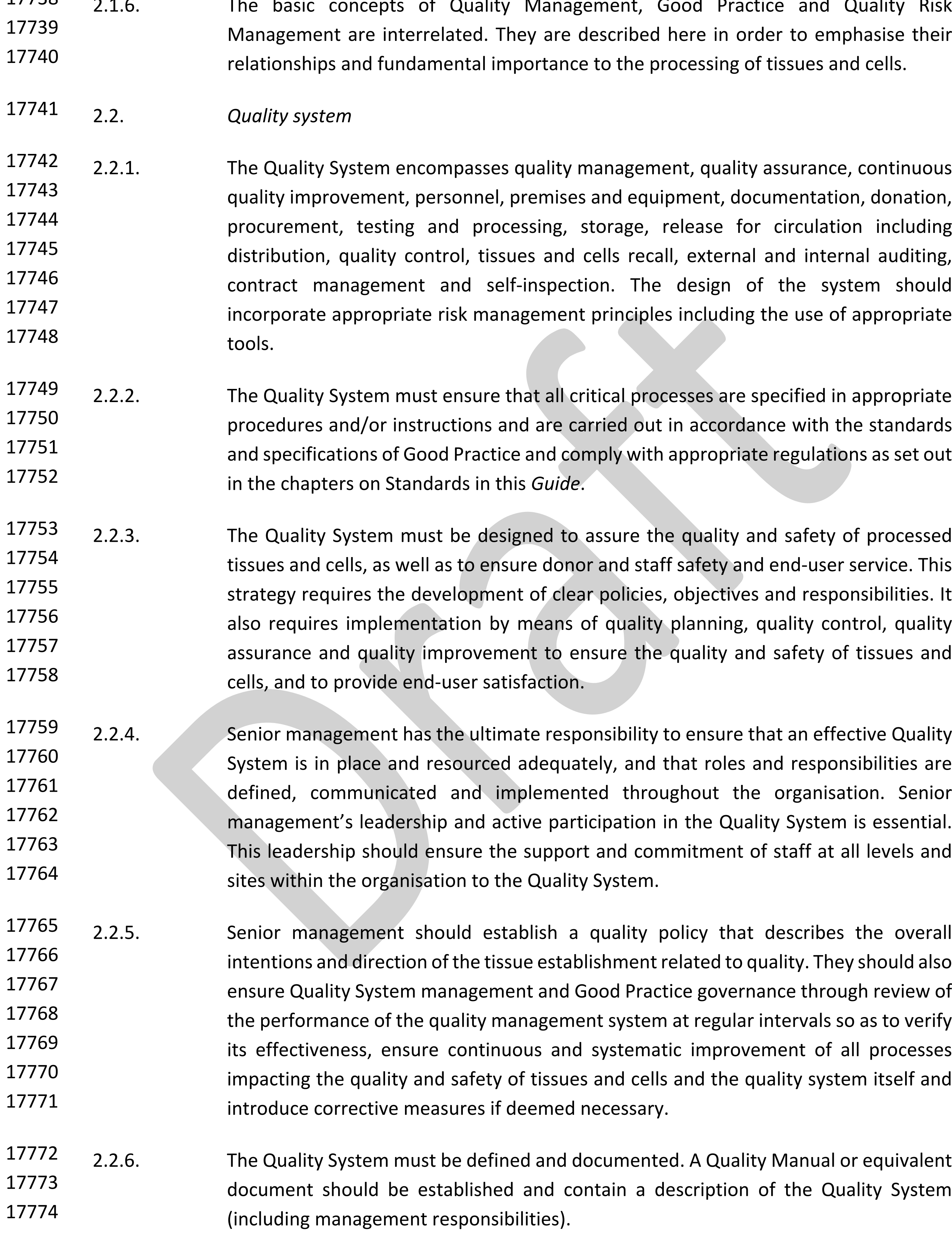

17738 2.1.6. The basic concepts of Quality Management, Good Practice and Quality Risk
17739 Management are interrelated. They are described here in order to emphasise their
17740 relationships and fundamental importance to the processing of tissues and cells.

17741 2.2. *Quality system*

17742 2.2.1. The Quality System encompasses quality management, quality assurance, continuous
17743 quality improvement, personnel, premises and equipment, documentation, donation,
17744 procurement, testing and processing, storage, release for circulation including
17745 distribution, quality control, tissues and cells recall, external and internal auditing,
17746 contract management and self-inspection. The design of the system should
17747 incorporate appropriate risk management principles including the use of appropriate
17748 tools.

17749 2.2.2. The Quality System must ensure that all critical processes are specified in appropriate
17750 procedures and/or instructions and are carried out in accordance with the standards
17751 and specifications of Good Practice and comply with appropriate regulations as set out
17752 in the chapters on Standards in this *Guide*.

17753 2.2.3. The Quality System must be designed to assure the quality and safety of processed
17754 tissues and cells, as well as to ensure donor and staff safety and end-user service. This
17755 strategy requires the development of clear policies, objectives and responsibilities. It
17756 also requires implementation by means of quality planning, quality control, quality
17757 assurance and quality improvement to ensure the quality and safety of tissues and
17758 cells, and to provide end-user satisfaction.

17759 2.2.4. Senior management has the ultimate responsibility to ensure that an effective Quality
17760 System is in place and resourced adequately, and that roles and responsibilities are
17761 defined, communicated and implemented throughout the organisation. Senior
17762 management's leadership and active participation in the Quality System is essential.
17763 This leadership should ensure the support and commitment of staff at all levels and
17764 sites within the organisation to the Quality System.

17765 2.2.5. Senior management should establish a quality policy that describes the overall
17766 intentions and direction of the tissue establishment related to quality. They should also
17767 ensure Quality System management and Good Practice governance through review of
17768 the performance of the quality management system at regular intervals so as to verify
17769 its effectiveness, ensure continuous and systematic improvement of all processes
17770 impacting the quality and safety of tissues and cells and the quality system itself and
17771 introduce corrective measures if deemed necessary.

17772 2.2.6. The Quality System must be defined and documented. A Quality Manual or equivalent
17773 document should be established and contain a description of the Quality System
17774 (including management responsibilities).

17775 2.2.7. All tissue establishments should be supported by an independent quality assurance

17776 17777 17778 function for fulfilling quality assurance. That function must be involved in all quality-related matters, and must review and approve all appropriate quality-related documents.

17779 17780 17781 17782 2.2.8. All procedures, premises and equipment that have an influence on the quality and safety of tissues and cells and tissues and cells components must be validated / qualified before introduction and must be re-validated at defined intervals, as determined on the basis of quality risk management.

17783 17784 17785 17786 2.2.9. A general policy regarding qualification of facilities and equipment as well as validation of processes, automated systems and laboratory tests must be in place. The formal objective of qualification / validation is to ensure compliance with the intended use and regulatory requirements.

17787 2.3. Change Control

17788 17789 17790 17791 17792 17793 2.3.1. A formal change control system must be in place to describe the actions to be taken to plan, evaluate and document any planned change which is proposed for range and/or specifications of both procured or processed tissues and cells, processes, equipment, environment (or site), method of processing or testing or any other change that may affect the reproducibility of a process, the quality and safety of tissues and cells, donors or recipients/patients.

17794 17795 17796 17797 17798 2.3.2. Change control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a tissues and cells product of the desired quality, consistent with the approved specifications. Supporting data, e.g. copies of documents, should be reviewed to confirm that the impact of the change has been demonstrated prior to final approval.

17799 17800 17801 2.3.3. The potential impact of a proposed change should be evaluated, and the degree of revalidation or additional testing, qualification and validation needed should be determined based on the principles of quality risk management.

17802 17803 17804 2.3.4. Changes should be authorised and approved by the responsible persons or relevant functional personnel in accordance with the tissues and cells establishment's quality system.

17805 17806 17807 2.3.5. After implementation of any change, an evaluation should be undertaken to confirm the quality objectives were achieved and that there was no unintended deleterious impact.

17808 17809 2.3.6. Where temporary and time limited changes are implemented, provisions should be in place to ensure and verify the changes are reversed as appropriate.

17810 17811 2.3.7. All changes should be evaluated for the requirement of notification to, or approval from a national health authority.

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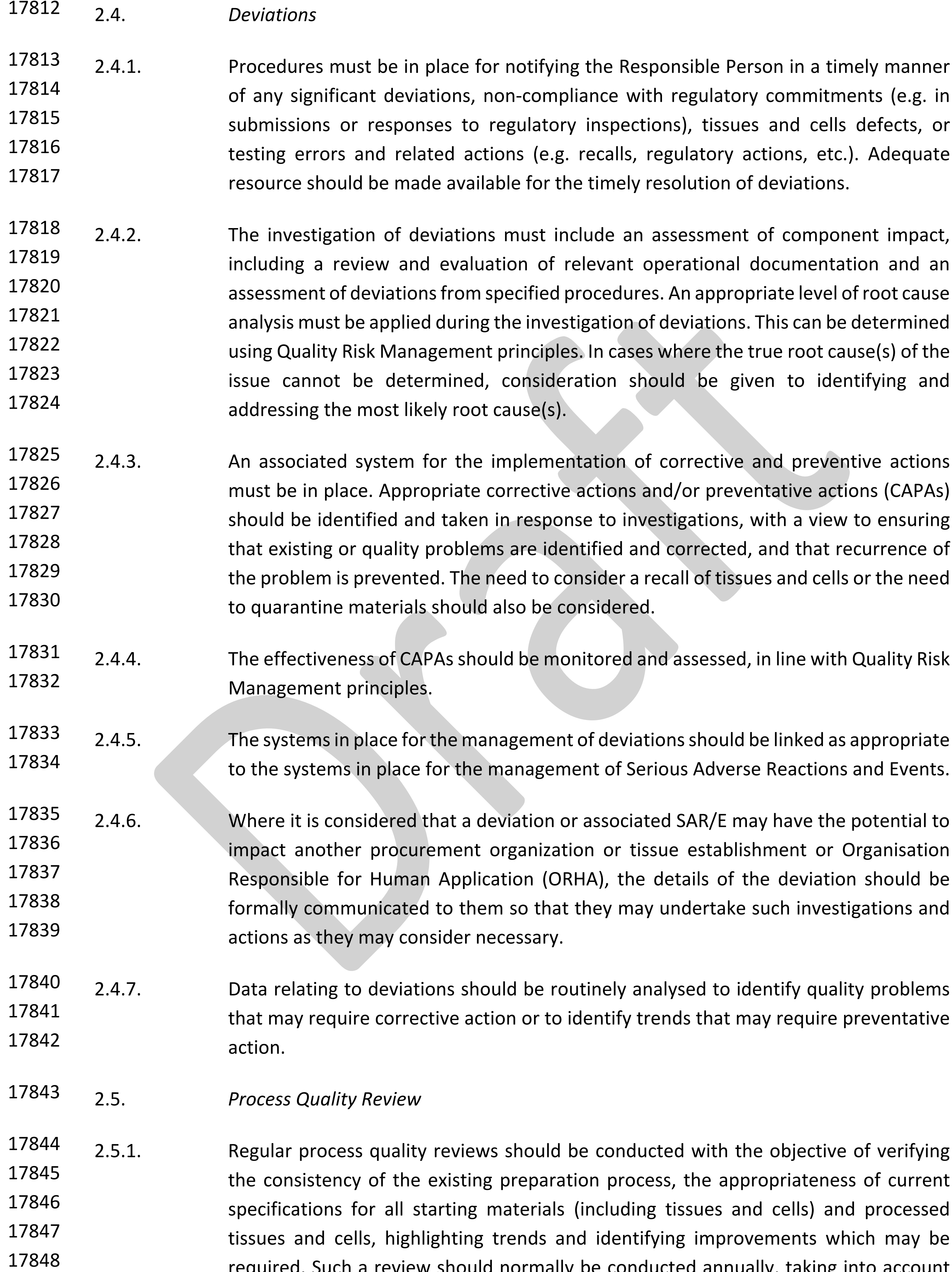

2.4. *Deviations*

2.4.1. Procedures must be in place for notifying the Responsible Person in a timely manner of any significant deviations, non-compliance with regulatory commitments (e.g. in submissions or responses to regulatory inspections), tissues and cells defects, or testing errors and related actions (e.g. recalls, regulatory actions, etc.). Adequate resource should be made available for the timely resolution of deviations.

2.4.2. The investigation of deviations must include an assessment of component impact, including a review and evaluation of relevant operational documentation and an assessment of deviations from specified procedures. An appropriate level of root cause analysis must be applied during the investigation of deviations. This can be determined using Quality Risk Management principles. In cases where the true root cause(s) of the issue cannot be determined, consideration should be given to identifying and addressing the most likely root cause(s).

2.4.3. An associated system for the implementation of corrective and preventive actions must be in place. Appropriate corrective actions and/or preventative actions (CAPAs) should be identified and taken in response to investigations, with a view to ensuring that existing or quality problems are identified and corrected, and that recurrence of the problem is prevented. The need to consider a recall of tissues and cells or the need to quarantine materials should also be considered.

2.4.4. The effectiveness of CAPAs should be monitored and assessed, in line with Quality Risk Management principles.

2.4.5. The systems in place for the management of deviations should be linked as appropriate to the systems in place for the management of Serious Adverse Reactions and Events.

2.4.6. Where it is considered that a deviation or associated SAR/E may have the potential to impact another procurement organization or tissue establishment or Organisation Responsible for Human Application (ORHA), the details of the deviation should be formally communicated to them so that they may undertake such investigations and actions as they may consider necessary.

2.4.7. Data relating to deviations should be routinely analysed to identify quality problems that may require corrective action or to identify trends that may require preventative action.

2.5. *Process Quality Review*

2.5.1. Regular process quality reviews should be conducted with the objective of verifying the consistency of the existing preparation process, the appropriateness of current specifications for all starting materials (including tissues and cells) and processed tissues and cells, highlighting trends and identifying improvements which may be required. Such a review should normally be conducted annually, taking into account

17849 the conclusions of previous reviews, be documented and include all tissues and cells
17850 which are imported, exported or intended for use in the manufacture of other
17851 products. Quality reviews may be grouped by tissues/cells type, where scientifically
17852 justified.

17853 2.5.2. A quality review of tissues and cells ready-for-circulation may also be considered as an
17854 instrument for surveying the overall quality status of a tissues and cells processing,
17855 including the procurement. It may include:

17856 2.5.2.1. review of starting materials, including tissues and cells;

17857 2.5.2.2. review of critical in-process controls;

17858 2.5.2.3. review of results of quality control and quality monitoring;

17859 2.5.2.4. review of all changes;

17860 2.5.2.5. review of the qualification status of equipment;

17861 2.5.2.6. review of technical agreements and contracts;

17862 2.5.2.7. review of all significant deviations and the CAPAs implemented;

17863 2.5.2.8. review of the findings of internal and external audits and inspections, and the CAPAs
17864 implemented;

17865 2.5.2.9. review of complaints and recalls;

17866 2.5.2.10. review of donor acceptance criteria;

17867 2.5.2.11. review of donor deferrals;

17868 2.5.2.12. review of look-back cases.

17869 2.5.3. The results of process quality reviews should be evaluated, and an assessment should
17870 be made whether CAPA or any revalidation should be undertaken. Reasons for such
17871 CAPA should be documented. Agreed CAPAs should be completed in a timely and
17872 effective manner. There should be management procedures for the ongoing
17873 management and review of these actions and the effectiveness of these procedures
17874 should be verified during self-inspection.

17875 2.6. *Good practice*

17876 2.6.1. Good Practice is the part of Quality Management that ensures that tissues and cells
17877 are consistently processed and controlled according to quality standards appropriate
17878 to their intended use. Good Practice is concerned with donation, procurement,
17879 processing, preservation, storage (hereinafter included in the generic term

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17880 17881 'preparation'), import, release for circulation including distribution, and quality control. The basic requirements are:

17882 17883 17884 2.6.1.1. All processes are defined clearly and reviewed systematically in the light of experience and shown to be capable of consistently delivering tissues and cells of the required quality and complying with their specifications. This strategy includes ensuring that:

17885 2.6.1.1.1. critical steps and significant changes to the process are validated;

17886 2.6.1.1.2. all requirements are provided including:

17887 2.6.1.1.2.1. appropriately qualified and trained personnel;

17888 2.6.1.1.2.2. adequate premises and space;

17889 2.6.1.1.2.3. suitable equipment and services;

17890 2.6.1.1.2.4. correct materials, containers and labels;

17891 2.6.1.1.2.5. approved procedures and instructions;

17892 2.6.1.1.2.6. suitable storage and transport;

17893 17894 2.6.1.1.3. instructions and procedures are written in an instructional form in clear and unambiguous language, and are applicable specifically to the facilities provided;

17895 2.6.1.1.4. operators are trained to carry out procedures correctly;

17896 17897 17898 17899 2.6.1.1.5. records are made, manually and/or by recording instruments, during preparation which demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality of the tissues and cells was as expected;

17900 2.6.1.1.6. any significant deviations are fully recorded and investigated;

17901 17902 17903 2.6.1.1.7. records of preparation processes, storage, release for circulation, including distribution, that enable the complete history of the tissues and cells to be traced are retained in a comprehensible and accessible form;

17904 17905 2.6.1.1.8. the release for circulation (including distribution) of the tissues and cells minimises any risk to their quality;

17906 17907 2.6.1.1.9. a system is available to recall any tissues and cells (including those processed using a batch of critical materials that have been distributed or issued);

17908 17909 17910 2.6.1.1.10. complaints about tissues and cells are examined, the causes of quality defects investigated, and appropriate measures taken in respect of the defective tissues and cells components to prevent reoccurrence.

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17911 17912 17913 17914 17915 17916 2.6.1.2. Quality Control is the part of Good Practice that is concerned with sampling, specifications and testing, as well as with the organisation, documentation and release procedures which ensure that materials are not released for use in processing, and tissues and cells are not released for circulation, including distribution, until their quality has been judged to be satisfactory and that the necessary and relevant tests have been carried out. The basic requirements are:

17917 17918 17919 17920 2.6.1.2.1. adequate facilities, trained and qualified personnel and approved procedures are available for sampling, inspecting/testing starting materials including tissues and cells, packaging materials, intermediate components, and finished/ready-for-circulation tissues and cells and, if appropriate, for monitoring environmental conditions;

17921 17922 17923 2.6.1.2.2. samples of starting materials (including tissues and cells), packaging materials, intermediate and processed tissues and cells are taken by approved personnel and methods;

17924 2.6.1.2.3. test methods are validated;

17925 17926 17927 2.6.1.2.4. records are made, manually and/or by recording instruments, which demonstrate that all the required sampling, inspecting and testing procedures were actually carried out. Any deviations are recorded and investigated fully;

17928 17929 2.6.1.2.5. the processed tissues and cells comply with the specifications and are correctly labelled;

17930 17931 17932 2.6.1.2.6. records are made of the results of inspection, and that testing of materials, intermediate and processed tissues and cells are formally assessed against specifications;

17933 17934 2.6.1.2.7. no tissues and cells are released for circulation, including distribution, that do not comply with the requirements of the relevant authorisations.

17935 17936 17937 17938 17939 2.6.1.3. Rolling quality reviews of all tissues and cells (including export-only tissues and cells) should be conducted with the objective of continuously verifying the consistency of the existing process; appropriateness of current specifications for both starting materials and processed tissues and cells, to highlight any trends and to identify processed tissues and cells and process improvements.

17940 2.7. *Quality risk management*

17941 17942 17943 17944 2.7.1. A quality risk management approach, consisting of a systematic process for the assessment, control, communication and review of risks to quality across the lifecycle of tissues and cells, should be applied. Appropriate statistical tools should be used (where appropriate) in the assessment of ongoing process capability.

17945 2.7.2. It must be ensured that the risks inherent in the use and handling of biological material

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are identified and minimised, consistent with maintaining adequate quality and safety for the intended purpose of the tissues and cells. (Directive 2006/86/EC Annex I A.5). Donor selection, and tissues and cells procurement, processing, storage and distribution activities should therefore be subjected to a comprehensive risk assessment encompassing all the process steps with respect to the procedures, materials, tests, personnel and premises and equipment involved.

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2.7.3. All components of the risk management process should be linked to the authorised activities of the tissue establishment and all elements of the quality management system should incorporate the principles of Quality Risk Management.

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2.7.4. Risk assessments should be based on an analysis of the risks related to the application of the specific type(s) of tissues and cells and be undertaken with the primary objective of identifying, where relevant, all those factors which could result in cross contamination, contamination with adventitious agents, the transmission of disease or infectious agents, the transmission of inherited conditions, mix-ups, or render the tissues or cells clinically ineffective or harmful to the recipient/patient.

17961 2.7.5. Such risks may, for example, derive from but are not limited to:

17962 2.7.5.1. the sensitivity of donor screening protocols and tests;

17963 2.7.5.2. procurement procedures;

17964 2.7.5.3. biological properties of the procured tissues and cells;

17965 2.7.5.4. the absence of standardised quality control tests;

17966 2.7.5.5. the use of potentially infective or known infective materials;

17967 2.7.5.6. processing, storage and transport procedures and environment.

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2.7.6. Risk assessments and associated management plans should identify and describe the principal activities of the tissue establishment and the circumstances to which the different phases of the plan apply.

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2.7.7. Any evaluation of the risk to quality must be based on scientific knowledge, experience with the process and, ultimately, connected to protection of the donor and recipient/patient.

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2.7.8. The level of effort, formality and documentation of the quality risk management process should be commensurate with the level of risk.

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2.7.9. Risk mitigation strategies should be developed on the basis of prospective risk analysis in order to maximise the quality and safety of tissues and cells and to protect recipients/patients, personnel and the process itself, as well as other linked or proximal processes.

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17980 2.7.10. Risk management should serve as documentation of the rationale for key safety or
17981 quality related decisions, such as in the case of actions to be take in relation to
17982 deviations and to determine the eligibility of impacted tissues and cells for clinical use.

17983 2.7.11. All risk assessment and management plans should include documentation on:

17984 2.7.11.1. the scope / circumstances for conducting the assessment;

17985 2.7.11.2. the individuals assigned to the work programme;

17986 2.7.11.3. identification of the hazards associated with the scope / circumstances;

17987 2.7.11.4. an estimate of their severity and probability of occurrence;

17988 2.7.11.5. the risk analysis, evaluation and control measures for these hazards;

17989 2.7.11.6. the scientific grounds for acceptance / rejection of the decision;

17990 2.7.11.7. a rationale for the acceptability of the residual risk;

17991 2.7.11.8. a statement of acceptance or otherwise of the residual risk

17992 2.7.12. Risk management should be used to support decision-making regarding the specific
17993 qualification / validation activities that need to be performed. The associated risk
17994 assessment should highlight the critical points in the processes allowing the
17995 development of an appropriate validation plan.
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17997 2.7.13. Risk management principles and methodologies should be incorporated into staff
17998 training programmes.

17999 2.7.14. Exceptional release

18000 2.7.14.1. In exceptional circumstances, an ORHA may agree with a tissue establishment, or
18001 procurement organisation in the case of direct distribution, that tissues or cells which
18002 do not meet defined release criteria can be released for use in a specific recipient.
18003 Whereas Directives 2004/23/EC, 2006/17/EC and 2006/86/EC lay down a number of
18004 specific requirements for performing a risk assessment when managing specified
18005 aspects of the quality and safety of tissues or cells for human use; exceptionally, and in
18006 circumstances where any other defined requirements of the Directives have not been
18007 complied with or cannot be complied with, and where clinical use of the impacted
18008 tissues and cells is required due to urgent medical need, the limited availability of
18009 alternative therapeutic options and the expected clinical benefit, a comprehensive
18010 documented risk assessment must be used to inform the decision of the Responsible
18011 Person as to whether the tissues or cells may be released for use. In such circumstances,
18012 the physician treating the intended recipient should work with the nominated registered
18013 medical practitioner who advises on and oversees the medical activities of the tissue
18014 establishment, in conducting the risk assessment and a risk–benefit analysis for the

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intended recipient. All associated discussions and conclusions must be documented and the treating physician must sign his/her agreement with the exceptional release and their acceptance of any implied risk for the intended recipient.

2.7.14.2. In the case of microbiological contamination of autologous tissues and cells or tissues and cells received from a specific allogenic donor, whereby a repeated procurement cannot be conducted or involves a high degree of risk; the risk assessment and risk–benefit analysis must be based on the nature and extent of the contamination and must specifically consider the risk based on identification of the contaminating micro-organisms and the potential for adequate prophylaxis of the intended recipient.

2.8. *Self-inspection, audits and improvements*

2.8.1. Self-inspection or audit systems must be in place for all elements of operations to verify compliance with the standards. They must be carried out regularly by trained and competent persons, in an independent way, and according to approved procedures.

2.8.2. All results must be documented and appropriate CAPAs must be implemented in a timely and effective manner.

3. Outsourced activities management (Contractual arrangements)

3.1. *General principles*

3.1.1. Outsourced activities that may impact on the quality and safety or efficacy of the tissues and cells must be correctly defined, agreed and controlled in order to avoid misunderstandings which could result in tissues and cells or work of unsatisfactory quality. There must be a written contract covering these activities, tissues and cells or processes to which they are related, and any technical arrangements made in connection with it.

3.1.2. All outsourced arrangements for tissue or cell procurement, processing and testing, including any proposed changes, must be done in accordance with a written contract, with reference to the specification for the tissues or cells concerned.

3.1.3. The responsibilities of each party must be clearly documented to ensure that Good Practice principles are maintained.

3.1.4. The contract giver is the establishment or institution that sub-contracts particular work or services to a different institution and is responsible for setting up a contract defining the duties and responsibilities of each party.

3.1.5. The contract acceptor is the establishment or institution that performs particular work or services under a contract for a different institution.

3.2. *The contract giver*

3.2.1. The contract giver is responsible for assessing the competence of the contract acceptor

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18053 18054 to successfully carry out the work being outsourced and for ensuring, by means of the contract, that the principles and guidelines of Good Practice are followed.

18055–18061 3.2.2. The contract giver must provide the contract acceptor with all the information necessary to carry out the contracted operations correctly and in accordance with the specification and any other legal requirements. The contract giver must ensure that the contract acceptor is fully aware of any problems associated with the materials, samples or the contracted processes that might pose a hazard to the premises, equipment, personnel, other materials or other tissues or cells of the contract acceptor.

18062–18065 3.2.3. The contract giver must ensure that all tissues or cells, analytical results and materials delivered by the contract acceptor comply with their specifications and that they have been released under a Quality System approved by the Responsible Person or other authorised person.

18066 3.3. *The contract acceptor*

18067–18069 3.3.1. The contract acceptor must have adequate premises, equipment, knowledge, experience and competent personnel to satisfactorily carry out the work requested by the contract giver.

18070 18071 3.3.2. The contract acceptor must ensure that all products, materials or test results delivered by the contract giver are suitable for their intended purpose.

18072–18076 3.3.3. The contract acceptor must not pass to a third party any of the work entrusted under the contract without the contract giver's prior evaluation and approval of the arrangements. Arrangements made between the contract acceptor and any third party must ensure that the relevant information is made available in the same way as between the original contract giver and contract acceptor.

18077 18078 3.3.4. The contract acceptor must refrain from any activity that may adversely affect the quality of the tissues or cells processed and/or analysed for the contract giver.

18079 3.4. *The contract*

18080–18084 3.4.1. A contract must be drawn up between the contract giver and the contract acceptor that specifies their respective responsibilities relating to the contracted operations. All arrangements for tissues or cells procurement, processing and testing must be in compliance with the requirements of Good Practice and regulatory requirements and agreed by both parties.

18085–18089 3.4.2. The contract must specify the procedure, including the necessary requirements to be provided by the contract acceptor, by which the Responsible Person or other authorised person releasing the tissues or cells can ensure that each component has been processed and/or distributed in compliance with the requirements of Good Practice and regulatory requirements.

18090–18095 3.4.3. The contract must clearly describe who is responsible for purchasing materials, testing and releasing materials, undertaking tissues or cells procurement, and for processing and testing (including in-process controls). In the case of sub-contracted analyses, the contract must state the arrangements for the collection / procurement of samples and the contract acceptor must agree that they can be subject to inspections by the Health Authorities.

18096 18097 3.4.4. Processing and distribution records, including reference samples if relevant, must be kept by, or be available to, the contract giver. Any records relevant to assessment of

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the quality of the tissues or cells in the event of complaints or a suspected defect must be accessible and specified in the defect/recall procedures of the contract giver.

The contract must permit the contract giver to audit the facilities of the contract acceptor.

4. Personnel and organisation

4.1. Personnel must be available in sufficient numbers and with the necessary qualifications and experience to carry out the activities related to the procurement, testing, processing, storage and release for circulation including distribution of tissues and cells for human application and be trained and assessed as competent to perform their tasks.

4.2. Management has the ultimate responsibility to determine and provide adequate and appropriate resources (human, financial, materials, facilities and equipment) to implement and maintain the quality management system and continually improve its suitability and effectiveness through participate in management reviews. The responsibilities placed on any one individual should not be so extensive as to present any risk to quality.

4.3. There should be an organisation chart in which the relationship between key personnel are clearly shown in the managerial hierarchy.

4.4. All personnel should have up to date job descriptions, which clearly set out their tasks and responsibilities.

4.5. Personnel in responsible positions should have adequate authority to carry out their responsibilities. Their duties may be delegated to designated deputies of a satisfactory qualification level. There should be no gaps or unexplained overlaps in the responsibilities of those personnel concerned with the application of good practice.

4.6. Individual responsibilities must be clearly defined and their correct understanding by individuals should be assessed and recorded.

4.7. Personnel signature lists should be available.

4.8. All personnel must receive initial and continued training appropriate to their specific tasks. Training records must be maintained. Training programmes must be in place and must include the principles of good practice.

4.9. Training should be provided for all personnel whose duties take them into processing areas or into laboratories (including the technical, maintenance and cleaning personnel).

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18133 4.10. There should be written policies and procedures to describe the approach to training,
18134 including a record of training that has taken place, its contents, and an assessment its
18135 effectiveness.

18136 4.11. Personnel must be provided with initial/basic training, updated training as required
18137 when procedures change, or scientific knowledge develops, and adequate
18138 opportunities for relevant professional development. The training programme must
18139 ensure and document that each individual:

18140 4.11.1. has demonstrated competence in the performance of their designated tasks;

18141 4.11.2. has an adequate knowledge and understanding of the scientific/technical processes
18142 and principles relevant to their designated tasks;

18143 4.11.3. understands the organisational framework, quality system and health and safety rules
18144 of the establishment in which they work; and

18145 4.11.4. is adequately informed of the broader ethical, legal and regulatory context of their
18146 work.

18147 4.12. The contents of training programmes must be periodically assessed, and the
18148 competence of personnel evaluated regularly.

18149 4.13. Only personnel who are authorised by defined procedures and documented as such
18150 may be involved in the procurement, processing, testing and distribution processes,
18151 including quality control and quality assurance.

18152 4.14. There must be written safety and hygiene instructions in place, adapted to the
18153 activities to be carried out.

18154 4.15. It is the organisation’s responsibility to provide instructions on hygiene and health
18155 conditions that can be of relevance to the quality of tissues and cells (e.g. during
18156 procurement) and to ensure that staff report relevant health problems. These
18157 procedures should be understood and followed in a strict way by all staff members
18158 whose duties take them into the processing and laboratory areas. Personnel should be
18159 instructed to use the hand-washing facilities.

18160 4.16. Steps should be taken to ensure as far as is practicable that no person affected by an
18161 infectious disease or having open lesions on the exposed surface of the body is
18162 engaged in the processing of tissues and cells. Medical examinations should be carried
18163 out when necessary to assure fitness for work and personal health. There should be
18164 instructions ensuring that health conditions that can be of relevance to the quality of
18165 tissues and cells are reported by the personnel.

18166 4.17. Visitors or untrained personnel should, preferably, not be taken into the procurement,
18167 processing and laboratory areas. If this is unavoidable, they should be given

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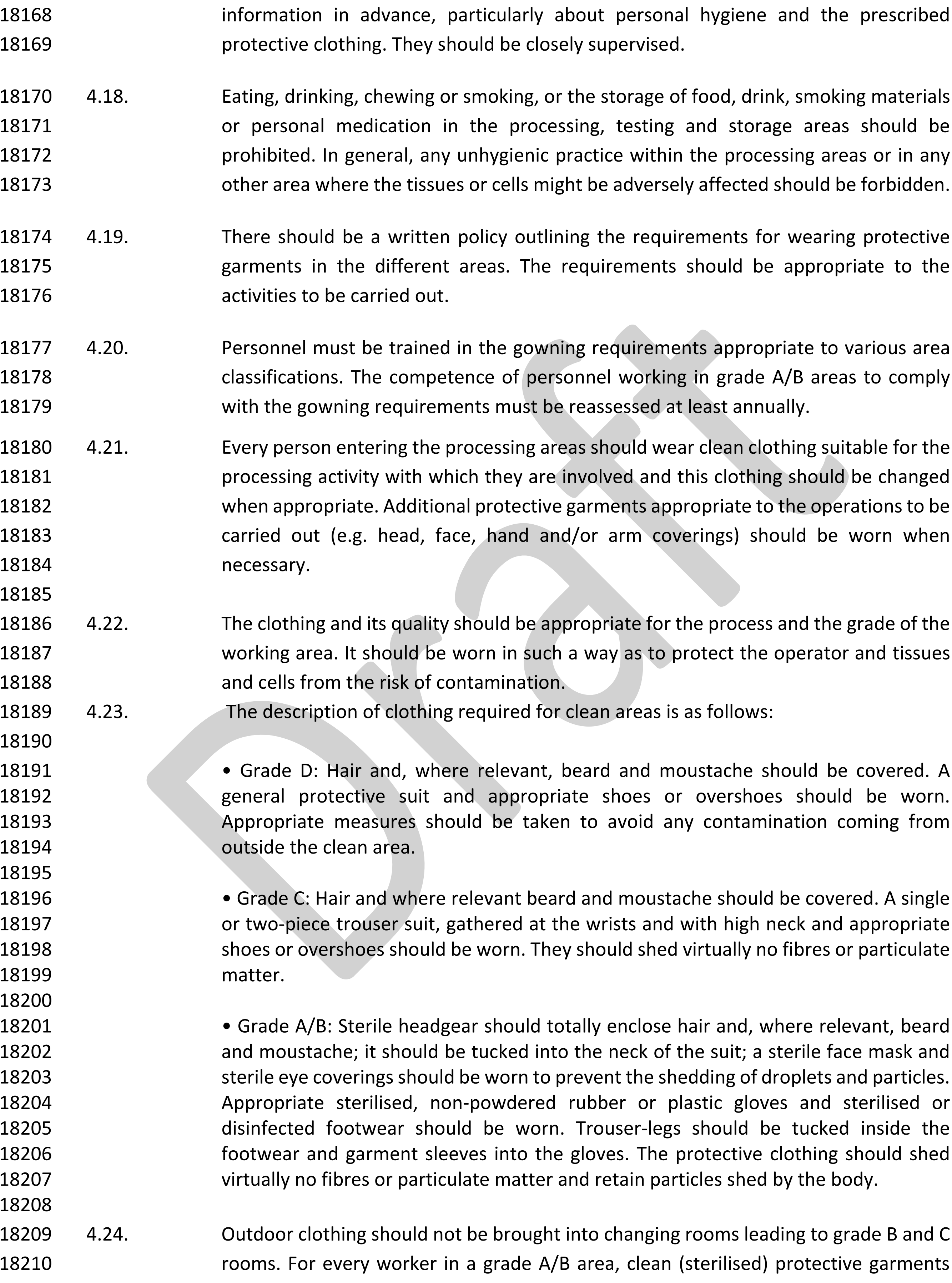
information in advance, particularly about personal hygiene and the prescribed protective clothing. They should be closely supervised.

4.18. Eating, drinking, chewing or smoking, or the storage of food, drink, smoking materials or personal medication in the processing, testing and storage areas should be prohibited. In general, any unhygienic practice within the processing areas or in any other area where the tissues or cells might be adversely affected should be forbidden.

4.19. There should be a written policy outlining the requirements for wearing protective garments in the different areas. The requirements should be appropriate to the activities to be carried out.



4.20. Personnel must be trained in the gowning requirements appropriate to various area classifications. The competence of personnel working in grade A/B areas to comply with the gowning requirements must be reassessed at least annually.

4.21. Every person entering the processing areas should wear clean clothing suitable for the processing activity with which they are involved and this clothing should be changed when appropriate. Additional protective garments appropriate to the operations to be carried out (e.g. head, face, hand and/or arm coverings) should be worn when necessary.

4.22. The clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way as to protect the operator and tissues and cells from the risk of contamination.

4.23. The description of clothing required for clean areas is as follows:

- Grade D: Hair and, where relevant, beard and moustache should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.

- Grade C: Hair and where relevant beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.

- Grade A/B: Sterile headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a sterile face mask and sterile eye coverings should be worn to prevent the shedding of droplets and particles. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser-legs should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

4.24. Outdoor clothing should not be brought into changing rooms leading to grade B and C rooms. For every worker in a grade A/B area, clean (sterilised) protective garments

(including face masks and eye coverings7) should be provided every time there is an entry into the clean area; the need to exit and re-enter the clean area for a different processing step/different batch should be determined by the risk of the activity. Gloves should be regularly disinfected during operations. Upon exit from a clean area there should be a visual check of the integrity of the garment.

4.25. Clean area clothing should be cleaned and handled in such a way that it does not gather additional contaminants which can later be shed. When working in a contained area, protective clothing should be discarded before leaving the contained area

4.26. Personnel working in clean areas must be given specific training on aseptic processing, including the basic aspects of microbiology.

4.27. Particular attention must be given to the qualification of the aseptic technique of personnel working in Grade A environments with Grade B backgrounds. Prior to participating in routine aseptic processing operations, personnel should be qualified through participation in successful process simulation tests. The usual approach is to conduct simulated processes using culture medium in place of, or added to, tissues or cells. Each operator should be qualified by performing three consecutive media simulation processes and thereafter should be requalified twice per year.

4.28. Microbial monitoring of personnel working in A/B areas should be performed after critical operations and when leaving the A/B area. A system of disqualification of personnel should be established based on the results of the monitoring program, as well as other parameters that may be relevant. Once disqualified, retraining/requalification is required before the operator can be involved in aseptic operations. It is advised that the retraining/requalification includes participation in a successful process simulation test.

5. Premises

5.1. *General requirements*

5.1.1. Premises must be suitable for carrying out the intended procedures in order to prevent errors (e.g. mix-ups, contamination, cross-contamination and improper labelling of tissues and cells).

5.1.2. Environmental conditions such as lighting, temperature, humidity and ventilation should be appropriate and controlled to assure safety and comfort to patients, donors, personnel and the accurate functioning of equipment during processing and storage.

18247 5.1.3. There must be adequate equipment and materials for the activities performed.

18248 5.1.4. Premises must be secure to prevent the entrance of unauthorized people and should
18249 not be used by right of way by personnel who do not work in it.

18250 5.1.5. Facilities should have appropriate design to permit ease of maintenance and cleaning.
18251 Cleaning and sanitation must be performed on a regular basis and documented. The
18252 efficacy of the methods used must be validated and monitored.

18253 5.1.6. A written safety manual and personal protective equipment must be available to
18254 minimize the risks to the health of personnel and visitors.

18255 5.1.7. All waste generated by the facilities must be disposed of in accordance with applicable
18256 laws and regulations.

18257 5.2. *Donor area*

18258 5.2.1. There must be a suitable space for the confidential interviews of living donors or the
18259 relatives of deceased donors and for physical examination of the donor.

18260 5.3. *Procurement area*

18261 5.3.1. The facility must be of adequate size to allow proper operations and ensure donor
18262 privacy and anonymity.

18263 5.3.2. Facilities in which tissues or cells are procured must meet appropriate grades of air
18264 quality and cleanliness. The appropriate standard of cleanliness will depend on the
18265 type of tissues or cells being procured, the degree of exposure of the tissues or cells
18266 during the procurement process, and the decontamination or sterilisation processes
18267 that will subsequently be applied to the tissues or cells during processing.

18268 5.3.3. The procurement facility should be divided in different areas of adequate size to
18269 prevent improper labelling and packaging, mix-ups and cross-contamination of tissues
18270 and cells.

18271 5.3.4. Tissues and cells procurement must be carried out in an appropriately equipped area
18272 for the initial treatment of donors experiencing adverse reactions associated with the
18273 donation. Access to an intensive care unit and or emergency service must be available,
18274 where applicable.

18275 5.4. *Processing area*

18276 5.4.1. The adequacy of the processing and in-process storage areas should permit the orderly
18277 and logical positioning of equipment and materials so as to minimise the risk of cross-
18278 contamination and to minimise the risk of errors or omission or wrong application of
18279 any of the processing or control steps.

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18280 18281 18282 18283 18284 18285 18286 5.4.2. Processing of tissues and cells exposed to the environment, without a subsequent microbial inactivation process, must take place in an environment with specified air quality with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current European Guide to Good Manufacturing Practice (GMP), Annex I and Directive 2003/94/EC and with a background environment appropriate for the processing of the tissues and cells concerned, but at least equivalent to GMP Grade D in terms of particles and microbial counts.

18287 18288 18289 18290 18291 5.4.2.1. While Grade D is specified as the minimum background environment, the actual background environment which is utilised must be selected and justified on the basis of an evaluation of the risks associated with the processing, testing and implantation of the types of tissues and cells concerned. Some national requirements may specify Grade C or B backgrounds for certain processes or types of tissue or cell.

18292 18293 18294 18295 18296 18297 18298 18299 5.4.2.2. A less stringent processing environment may be acceptable where a validated microbial inactivation or validated terminal sterilisation process is applied or where it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissue or cell or, where it is demonstrated that the mode and route of application of the tissue or cell to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with cell and tissue transplantation or, where it is not technically possible to carry out the required process in a Grade A environment.

18300 18301 5.4.2.3. The risk assessment for determination of the processing environment must consider several factors such as:

• Tissue or cell contamination during open *versus* closed processing.
• Effectiveness of the processing method to remove contaminants.
• Suboptimal detection of contaminants due to the sampling method.
• Transfer of contaminants at transplantation.

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18303 18304 18305 18306 18307 The associated guidelines on environmental monitoring, relevant to the determined classification for the processing environment should be considered at least in the context of a risk-based analysis which takes full account of the specific protocols and risk mitigation strategies relevant to the risks associated with the processing, testing and implantation of the types of tissues and cells concerned.

18308 18309 5.4.3. Clean rooms and laminar flow cabinets must be classified, re-qualified and monitored in accordance with EN ISO14644 and EU GMP Annex 1.

18310 18311 18312 5.4.4. Critical facility parameters identified to be a risk to the tissues and cells, such as temperature, humidity, air-supply conditions, pressure differentials, particles number and microbial contamination must be checked, monitored and recorded.

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5.4.5. Environmental monitoring programs are an important tool by which the effectiveness of contamination control measures can be assessed. The environmental monitoring program should include an assessment of non-viable and viable contamination and air pressure differentials.

5.4.6. The monitoring locations should be determined having regard to the risks (e.g. at locations posing the highest risk of contamination) and the results obtained during the qualification of the premises.

5.4.7. The number of samples, volume, frequency of monitoring, alert and action limits should be appropriate taking into account the risks and the overall control strategy for the establishment. Sampling methods should not pose a risk of contamination to the processing activities.

5.4.8. *Non-viable particulate monitoring*

5.4.8.1. Airborne particle monitoring systems should be established to obtain data for assessing potential contamination risks and to ensure maintenance of the designated environment in the clean room. Environmental monitoring is also expected for isolators and biosafety cabinets.

5.4.8.2. The degree of environmental control of non-viable particulate and the selection of the monitoring system should be adapted to the specific risks of tissues and cells and of the preparation process / processing (e.g. live organisms). The frequency, sampling volume or duration, alert limits and corrective actions should be established case by case having regard to the risks. It is not necessary for the sample volume to be the same as that used for qualification of the clean room

5.4.8.3. Appropriate alert and actions limits should be defined. With a view to identify potential changes that may be detrimental to the process, the alert limits for grades B to D should be lower than those specified as action limits and should be based on the area performance.

5.4.8.4. The monitoring system should ensure that when alert limits are exceeded, the event is rapidly identified (e.g. alarm settings). If action limits are exceeded, appropriate corrective actions should be taken. These should be documented.

5.4.8.5. The maximum permitted particle concentrations in accordance with Annex 1 of EU GMP are as follows:

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	Maximum permitted number of particles equal or greater than 0.5 µm		
	At rest (per m^3)	In operation (per m^3)	ISO classification (At rest/in operation)
Grade			
A	3 520	3 520	5/5
B	3 520	352 000	5/7
C	352 000	3 520 000	7/8
D	3 520 000	Not defined	8

18345 5.4.8.6. When the risk assessment (see 5.4.2.3) concludes that the most stringent air quality
18346 standard is required to achieve an acceptable level of risk, then normally grade A in
18347 full compliance with GMP for aseptic processing (i.e. with a grade B background,
18348 accessed via grades C and D) should be applied. This implies that particle monitoring
18349 should be undertaken for the full duration of critical processing, including equipment
18350 assembly, except where duly justified (e.g. contaminants in the process that would
18351 damage the particle counter, production of particles by a process itself, e.g. bone
18352 cutting or grinding or when this would present a hazard to the tissues or cells). In such
18353 cases, monitoring during equipment set-up operations should take place (i.e. prior to
18354 exposure of the tissues and cells to the hazard). For this most stringent standard,
18355 monitoring should also be performed during simulated operations.

18356 5.4.8.7. For grade B areas, there should be particle monitoring during critical operations, within
18357 the limitation referred to in 5.4.8.6, albeit the monitoring does not need to cover the
18358 entire duration of the critical processing. The grade B area should be monitored at an
18359 appropriate frequency and with suitable sample size to permit that changes in levels
18360 of contamination are identified.

18361 5.4.8.8. The monitoring strategy regarding grades C and D should be set having regard to the
18362 risks and in particular the nature of the operations conducted.

18363 5.4.8.9. When there is no critical operations on-going (i.e. at rest), sampling at appropriate
18364 intervals should be conducted. While at rest, the heating, ventilating and air-
18365 conditioning (HVAC) system should not be interrupted, as this may trigger the need for
18366 re-qualification. In the event of an interruption, a risk assessment should be conducted
18367 to determine any actions that may be required taking account of the activities
18368 performed in the affected areas (e.g. additional monitoring).

18369 5.4.8.10. While not required for qualification purposes, the monitoring of the ≥5.0 µm particle
18370 concentration in grade A and B areas is required for routine monitoring purposes as it
18371 is an important diagnostic tool for early detection of failures. While the occasional
18372 indication of ≥5.0 µm particle counts may be false counts, consecutive or regular
18373 counting of low levels is an indicator of a possible contamination and it should be
18374 investigated. Such events may, for example, be indicative of early failure of the HVAC

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18375 (heating, ventilation and air-conditioning system), filling equipment failure or may also
18376 be diagnostic of poor practices during machine set-up and routine operation.

18377 5.4.9. *Viable particle monitoring*

18378 5.4.9.1. Checks to detect the presence of specific microorganisms in the clean room (e.g. yeast,
18379 moulds, etc.) should be performed as appropriate. Viable particle monitoring is also
18380 expected for isolators and biosafety cabinets.

18381 5.4.9.2. Where aseptic operations are performed, monitoring should be frequent using
18382 methods such as settle plates, volumetric air and surface sampling (e.g. swabs and
18383 contact plates). Rapid microbial monitoring methods should be considered and may
18384 be adopted after validation of the premises.

18385 5.4.9.3. Continuous monitoring is required during critical operations where the tissues and
18386 cells are exposed to the environment. Surfaces and personnel should be monitored
18387 after critical operations. Additional microbiological monitoring may also be required
18388 outside processing operations depending on the risks.

18389 5.4.9.4. The following recommended maximum limits apply for microbiological monitoring of
18390 clean areas in accordance with Annex 1 of EU GMP:

18391

18392

Grade	Air sample cfu/m3	Settle plates (diameter 90mm) cfu/4 hours*	Contact plates (diameter 55 mm) cfu/plate
A**	<1	<1	<1
B	10	5	5
C	100	50	25
D	200	100	50

* Individual settle plates may be exposed for less than 4 hours. Where settle plates are exposed for less than 4 hours the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 hours.

18393 5.4.9.5. Appropriate alert and actions limits should be defined. With a view to identify potential
18394 changes that may be detrimental to the process, the alert limits for grades B to D
18395 should be lower than those specified as action limits and should be based on the area

18396 5.4.9.6. If microorganisms are detected in a grade A area, they should be identified to species
18397 level and the impact thereof on tissues and cells quality and on the suitability of the
18398 premises for the intended operations should be assessed.

18399 5.4.10. *Air pressure*

18400 5.4.10.1. An essential part of contamination prevention is the adequate separation of areas of
18401 operation. To maintain air quality, it is important to achieve a proper airflow from
18402 areas of higher cleanliness to adjacent less clean areas. It is fundamental for rooms of
18403 higher air cleanliness to have a substantial positive pressure differential relative to
18404 adjacent rooms of lower air cleanliness. These pressure cascades should be clearly

18405 defined and continuously monitored with appropriate methods (e.g. alarm settings).
18406 Adjacent rooms of different grades should have a pressure differential of 10-15 Pa
18407 (guidance values).

18408 5.4.10.2. Negative pressure may be required in specific areas for containment reasons (e.g.
18409 handling of viral positive material). In such cases, the negative pressure areas should
18410 be surrounded by a positive pressure clean area of appropriate grade.

18411 5.5. *Storage area*

18412 5.5.1. Storage rooms must be located in a secure area and access must be limited to
18413 authorized personnel.

18414 5.5.2. Storage areas should be of appropriate size to allow orderly storage of materials,
18415 reagents and of tissues and cells.

18416 5.5.3. Dedicated areas must be available for storing tissues and cells in quarantine, and /or
18417 for storing unqualified materials.

18418 5.5.4. Storage areas for tissues and cells should be maintained within defined temperature
18419 limits. Where special storage conditions are required (e.g. temperature, humidity)
18420 these must be checked, monitored and recorded.

18421 5.5.5. An alarm system should be in place to alert users in a timely manner to any deviation
18422 from the predefined storage conditions. Alarm systems placed in storage devices must
18423 be continuously active and able to alert personnel on a 24-hour basis.

18424 5.5.6. Provisions must be in place in the event of equipment or power failure.

18425 5.5.7. Oxygen sensors must be appropriately placed in areas where liquid nitrogen is present.

18426 5.6. *Ancillary areas*

18427 5.6.1. Suitable facilities for changing clothes and for washing hands should be readily
18428 accessible.

18429 5.6.2. Staff rest and refreshment areas should be separate from other rooms.

18430 5.6.3. Archive store and administrative areas should be protected against unauthorised
18431 access to ensure that records and documents are maintained in a confidential manner
18432 as required by applicable laws and regulations.

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18435
6. Equipment and materials

18436
6.1. General requirements

18437–18438 6.1.1. Tissue Establishments must have equipment and materials appropriate to the activities for which they are authorised.

18439–18441 6.1.2. All equipment must be *designed, located,* qualified, calibrated and maintained to suit its intended purpose and comply with the general safety requirements of this Guide and the specific requirement relevant to the type(s) of tissues and cells.

18442–18444 6.1.3. Equipment with an appropriate range and precision for measuring, weighing, recording and control should be available and be calibrated and checked at defined intervals using appropriate methods.

18445–18447 6.1.4. All critical equipment and technical devices must be identified and qualified, regularly inspected and preventively maintained in accordance with the manufacturers' instructions.

18448–18452 6.1.5. Where equipment or materials affect critical processing or storage parameters (e.g. temperature, pressure, particle counts, microbial contamination levels), they must be identified and must be the subject of appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times.

18453–18457 6.1.6. A temperature monitoring system should be utilised to document temperatures and to alert staff when temperatures have deviated from acceptable limits. Procedures should be in place for reviewing temperatures. If storage utilises liquid nitrogen, either liquid nitrogen levels or temperature should be monitored and documented at an interval specified in the SOP and determined by validation.

18458–18460 6.1.7. Procedures for the operation of each piece of critical equipment, detailing the action to be taken in the event of malfunctions or failure, must be available and appropriate records kept.

18461–18463 *6.1.8.* The services that could impact on the tissues/cells quality (i.e. compressed air, heating, ventilating and air conditioning) should be qualified and scheduled in a maintenance programme.

18464–18465 6.1.9. Equipment must be selected to minimise any hazard to donors, personnel or tissues and cells.

18466–18469 6.1.10. All validated processes must use qualified equipment. Qualification results must be documented. Regular maintenance and calibration must be carried out and documented according to established procedures. The qualification and maintenance status of each item of equipment must be available.

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18470 18471 18472 18473
6.1.11. All critical equipment must have regular, planned maintenance to detect or prevent avoidable errors and keep the equipment in its optimum functional state. The maintenance intervals and actions must be determined for each item of equipment and should be at least in accordance with those specified by the manufacturer.

18474 18475
6.1.12. Records of maintenance activities should be clear and comprehensible and detail the specific activities performed as part of maintenance.

18476 18477
6.1.13. New and repaired equipment must meet qualification requirements when installed and must be qualified before use. Test results must be documented.

18478 18479 18480 18481
6.1.14. All modifications, enhancements or additions to qualified systems and equipment must be managed through the change control procedure of the tissue establishment. The effect of each change to the system or equipment, as well as its impact on quality and safety, must be determined to identify the extent of revalidation required.

18482 18483
6.1.15. Instructions for use, maintenance, servicing, cleaning, disinfection and sanitation must be available. These activities must be performed regularly and recorded accordingly.

18484 18485
6.1.16. Repair and maintenance operations should not present any hazard to the donor, staff or quality of the tissues and cells and tissues and cells components.

18486 18487 18488
6.1.17. Equipment should be designed or selected so that it can be thoroughly cleaned and, where necessary, decontaminated. This should be performed according to detailed and written procedures. It should be stored only in a clean and dry condition.

18489 18490
6.1.18. Washing/cleaning solutions and equipment should be chosen and used so that they are not sources of contamination or toxicity.

18491 18492
6.1.19. Equipment should be installed in such a way as to prevent any risk of error or of contamination.

18493 18494
6.1.20. Fixed pipework should be clearly labelled to indicate the contents and, where applicable, the direction of flow.

18495 18496 18497
6.1.21. Distilled, deionized and, where appropriate, other water pipes should be sanitised according to written procedures that detail the action limits for microbiological contamination and the measures to be taken.

18498
6.2. Calibration and monitoring of equipment

18499 18500 18501 18502 18503
6.2.1. A mechanism to ensure the adequacy of the calibration and monitoring programmes should be established, and qualified personnel are available for their implementation. A calibration and monitoring plan should be used to define the requirements for establishing and implementing a calibration programme that includes the frequency of monitoring.

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6.2.2. All equipment with a critical measuring function must be calibrated against a traceable standard if available. Adequate records of such tests should be maintained, including the values obtained prior to any adjustment. Calibration reports should include the accuracy of any testing equipment and traceability to a national standard. The report and/or calibration certificate must be reviewed and signed to show acceptance of the document. Any failed calibrations require investigation of the potential impact.

6.2.3. Trending and analyses of calibration and monitoring results should be a continuous process. Intervals of calibration and monitoring should be determined for each item of equipment to achieve and maintain a desired level of accuracy and quality. The calibration and monitoring procedure should be based on a recognised international standard. The calibration status of all equipment that requires calibration should be readily available.

6.2.4. To ensure appropriate performance of a system or equipment, a monitoring plan should be developed and implemented. The plan should take into account the criticality of the system or equipment, and should outline monitoring, user-notification and problem-resolution mechanisms. When appropriate, equipment should be subject to continuous monitoring linked to an alarm system. If an unusual event is observed, personnel should follow the standard response described in the monitoring plan. The standard response should involve the notification of affected personnel and, where appropriate, initiation of a resolution response to the problem and risk assessment of the affected tissues and cells. Depending on the severity of the problem and the criticality of the system or equipment, a back-up plan should be in place and be implemented to keep the process or system operating.

6.2.5. The ability of a supplier to maintain its activities relating to a system or equipment must be re-qualified on a regular basis; notably to anticipate weaknesses in services or to manage changes in the system, equipment or supplier. The periodicity and detail of the re-qualification process should be linked the level of risk of using the system or equipment and should be planned for each supplier.

6.2.6. Defective equipment should be labelled clearly as such and, if possible, removed from processing areas

6.3. Data processing systems

6.3.1. If computerised systems are used, software, hardware and back-up procedures should be validated/qualified before use, be checked regularly to ensure reliability, and be maintained in a validated/qualified state. Hardware and software must be protected against unauthorised use or unauthorised changes. The back-up procedure must prevent loss of or damage to data at expected and unexpected down-times or function failures.

6.3.2. Systems must be properly maintained at all times. Documented maintenance plans

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18542 must be developed and implemented.

18543 6.3.3. Changes in computerised systems must be validated; applicable documentation must
18544 be revised, and relevant personnel trained appropriately before any critical change is
18545 introduced into routine use. Computerised systems must be maintained in a validated
18546 /qualified state. This must include user-testing to demonstrate that the system is
18547 correctly performing all specified functions both at initial installation and after any
18548 system modifications.

18549 6.3.4. There must be a hierarchy of permitted user access to enter, amend, read or print data.
18550 Methods of preventing unauthorised entry must be in place, such as personal identity
18551 codes or passwords that are changed regularly.

18552 6.3.5. All necessary measures must be taken to ensure protection of data. These measures
18553 must ensure that safeguards against unauthorised additions, deletions or
18554 modifications of data and transfer of information are in place to resolve data
18555 discrepancies, and to prevent unauthorised disclosure of such information.

18556 6.3.6. Computer systems designed to control decisions related to inventories and release of
18557 tissues and cells should prevent the release of all tissues and cells considered not
18558 acceptable for release. Preventing release of any tissues and cells from a future
18559 donation from a deferred donor should be possible.

18560 *6.4. Equipment and Materials for Procurement and Processing and Storage*

18561 6.4.1. Critical equipment and materials should meet documented requirements and
18562 specifications and when applicable the requirements of Council Directive 93/42/EEC
18563 of 14 June 1993 concerning medical devices (1) and Directive 98/79/EC of the
18564 European Parliament and of the Council of 27 October 1998 on in vitro diagnostic
18565 medical devices (2). (Directive 2006/86/EC Annex I C.6). In this context, 'critical' means
18566 those equipment and materials that come in contact with the tissues or cells or
18567 influence the critical quality/safety attributes of the tissues and cells directly (e.g. an
18568 additive) or indirectly (e.g. donor testing kits).

18569 6.4.2. Procurement, processing and storage equipment must be managed in accordance with
18570 the standards and specifications laid down in the directives and with due regard to
18571 relevant national and international regulation, standards and guidelines covering the
18572 sterilisation of medicines and medical devices. Qualified, sterile instruments and
18573 procurement devices must be used for tissue and cell procurement. The appropriate
18574 equipment and instruments should be used, in order to guarantee the quality of the
18575 specific recovered tissue.

18576 6.4.3. Wherever possible, only CE marked medical devices should be used and all concerned
18577 staff must have received appropriate training on the use of such devices.

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18578 6.4.4. Sterile instruments and devices must be used for tissue and cell procurement.
18579 Instruments or devices must be of good quality, validated or specifically certified and
18580 regularly maintained for the procurement of tissues and cells.

18581 6.4.5. When reusable instruments must be used, a validated cleaning and sterilisation
18582 procedure for removal of infectious agents should be in place.

18583 6.4.6. Materials and parts of equipment that come into contact with tissues and cells must
18584 not be reactive, additive or absorptive to such an extent that they affect the quality of
18585 the tissues and cells and thus present any hazard.

18586 6.4.7. A standardized written procedure (SOP) must be in place to regulate the specific
18587 materials that come into contact with tissues and cells during processing, the addition
18588 of therapeutic products to tissues and cells, the choice of those media and products,
18589 their characteristics, their source and control and the associated requirements for
18590 asepsis and labelling. A procedure to select the materials must be in place.

18591 6.4.8. A controlled list should be constructed of all materials that come into contact with the
18592 tissues or cells or that influence the quality or safety of the tissues or cells. Detailed
18593 specifications for such critical reagents and consumables must be documented.

18594 6.4.9. Only materials from qualified suppliers that meet the documented specifications
18595 should be used.

18596 6.4.10. Specifications for starting and primary or printed packaging materials should include,
18597 if applicable:

18598 6.4.10.1. a description of the materials, including; the designated name and the internal code
18599 reference; the reference, if any, to a pharmacopoeia monograph; the approved
18600 suppliers and, if possible, the original producer of the products; a specimen of printed
18601 materials.

18602 6.4.10.2. directions for sampling and testing or reference to procedures;

18603 6.4.10.3. qualitative and quantitative requirements with acceptance limits;

18604 6.4.10.4. storage conditions and precautions;

18605 6.4.10.5. the maximum period of storage before re-examination.

18606 6.4.11. When using processing media and / or added therapeutic products, their source, lot
18607 number and expiration date must be recorded in the relevant processing
18608 documentation.

18609 6.4.12. The following aspects should be verified with regard to materials used during the
18610 processing of tissues and cells:

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18611 6.4.12.1. Free of viral contamination (certificate should be available);

18612 6.4.12.2. Free of transmissible spongiform encephalopathy (TSE) contamination (certificate
18613 should be available);

18614 6.4.12.3. Produced under GMP conditions when available;

18615 6.4.12.4. For human use when available;

18616 6.4.12.5. Identity, purity, sterility and quantification of endotoxins should be defined;

18617 6.4.12.6. Human and animal origin reagents should be substituted when possible;

18618 6.4.12.7. Antibiotics should be avoided when possible;

18619 6.4.12.8. Final residues of reagents should be quantified when possible;

18620 6.4.12.9. Risk assessment of potential residues in the final tissues and cells.

18621 6.4.13. The specifications of the materials used to perform any evaluation of the donor should
18622 be described and these materials should not have any negative impact on the
18623 maintenance of the donors and reliability of the evaluation results.

18624 6.4.14. Only materials from approved suppliers that meet their documented requirements
18625 and specifications must be used. Batch acceptance testing or checking of each delivery
18626 of materials should be carried out and documented before release for use in tissue or
18627 cell procurement or processing. Critical materials must be released by a person
18628 qualified to perform this task.

18629 6.4.15. Manufacturers of sterile materials should provide a certificate of release for each
18630 batch. The tissue establishment should define acceptance criteria for such certificates
18631 in writing, and should include at least the name of the material, manufacturer,
18632 compliance with relevant requirements (e.g. pharmacopoeias or regulations for
18633 medical devices) and confirmation that the materials are sterile and pyrogen-free as
18634 appropriate.

18635 6.4.16. All incoming materials should be checked to ensure that the consignment corresponds
18636 to the order.

18637 6.4.17. The status of materials (quarantined, released, rejected) should be indicated clearly.

18638 6.4.18. Materials must be stored under the conditions established by the manufacturer and in
18639 an orderly manner that permits segregation by status, batch and lot as well as stock
18640 rotation.

18641 6.4.19. Storage and use of materials should follow the ‘first-in first-out’ principle (i.e. the
18642 material that entered storage first should be used first) taking into account the expiry
18643 date of materials.

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18644 *6.5. Control of equipment and materials*

18645 *6.5.1. General principles*

18646 6.5.2. All changes to equipment and materials should be managed in accordance with the
18647 principles of change control.

18648 6.5.3. Documented systems for purchasing equipment and materials should be available.
18649 These should identify the specific requirements for establishing and reviewing
18650 contracts for the supply of both equipment and materials.

18651 6.5.4. The contracting process should include:

18652 6.5.4.1. checks prior to awarding the contract to help ensure suppliers meet the organisation's
18653 needs;

18654 6.5.4.2. appropriate checks on received goods to confirm they meet specifications;

18655 6.5.4.3. the requirement for manufacturers to provide a certificate of analysis for critical
18656 material;

18657 6.5.4.4. checks to ensure that goods in use continue to meet specifications;

18658 6.5.4.5. regular contact with suppliers to help understand and resolve problems;

18659 6.5.4.6. performance of regular audits.

18660 6.5.5. Assessment of the performance of equipment should occur in the following situations:

18661 6.5.5.1. upon commissioning of new equipment, which must include design, installation,
18662 operational and performance qualifications;

18663 6.5.5.2. after any relocation, repairs or adjustments that might potentially alter equipment
18664 functioning;

18665 6.5.5.3. After any major repair or modification, the equipment or critical equipment should be
18666 checked and validated before its release;

18667 6.5.5.4. if ever a doubt arises that the equipment is not functioning appropriately.

18668

18669 7. Qualification and Validation

18670 7.1. *General*

18671 7.1.1. TEs should establish documented evidence that provides a high degree of assurance
18672 that a specific process, piece of equipment or environment will consistently produce

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18673 processed tissues and cells meeting pre-determined specifications and quality
18674 attributes.

18675 7.1.2. All critical equipment and technical devices must be identified and qualified.

18676 7.1.3. All critical processing procedures must be validated and must not render the tissues
18677 and cells clinically ineffective or harmful to the recipient.

18678 7.1.4. TEs should identify what validation work is needed to prove control of the critical
18679 aspects of their particular processes.

18680 7.1.5. Significant changes to the facilities, the equipment and the processes, which may
18681 affect the quality of the tissues and cells, should be qualified / validated.

18682 7.1.6. A risk assessment approach should be used to determine the scope and extent of
18683 validation / qualification. Such risk assessment should take into account all the
18684 equipment (e.g. autoclave, incubator, freeze drier), facilities (e.g. clean rooms, laminar
18685 flow module), electronic systems (e.g. clean rooms environmental monitoring system,
18686 tissues processing system) and processes (e.g. musculoskeletal processing, skin
18687 processing, clean rooms disinfection, tissue transport, analytical methods) which may
18688 impact on the quality of processed tissues and cells.

18689 7.1.7. The results from the risk assessment study regarding the scope of validation /
18690 qualification activities within a TE should be covered in a Validation Master Plan.

18691 7.2. *Documentation*

18692 7.2.1. The Validation Master Plan should consist of at least:

18693 7.2.1.1. description of the TE;

18694 7.2.1.2. list of equipment, facilities, electronic systems and processes that need to be qualified
18695 or validated;

18696 7.2.1.3. state of validation /qualification of each element within the scope;

18697 7.2.1.4. validation /qualification programme;

18698 7.2.1.5. validation /qualification activities responsibilities;

18699 7.2.1.6. procedures related to validation /qualification activities;

18700 7.2.1.7. criteria for requalification or revalidation;

18701 7.2.2. The activities of qualification or validation should be described in a protocol containing
18702 at least:

18703 7.2.2.1. objective;

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18704 7.2.2.2. scope;

18705 7.2.2.3. responsibilities;

18706 7.2.2.4. related documents;

18707 7.2.2.5. stages of qualification or validation;

18708 7.2.2.6. acceptance criteria.

18709 7.2.3. A validation / qualification report should be issued reflecting the results of the
18710 activities containing at least:

18711 7.2.3.1. objective;

18712 7.2.3.2. scope;

18713 7.2.3.3. responsibilities;

18714 7.2.3.4. related documents;

18715 7.2.3.5. deviations from the protocol;

18716 7.2.3.6. results;

18717 7.2.3.7. conclusions.

18718 7.3. *Facility, system and equipment qualification*

18719 7.3.1. *Qualification for new facilities, systems and equipment*

18720 7.3.1.1. The qualification of new facilities, systems or equipment begins with Design
18721 Qualification (DQ) and progresses successively through Installation Qualification (IQ),
18722 Operational Qualification (OQ) and Performance Qualification (PQ)

18723 7.3.1.1.1. DQ is the documented verification that the proposed design of the facilities,
18724 equipment, or systems is suitable for the intended purpose. During DQ the compliance
18725 of the design with good practice should be demonstrated and documented.

18726 7.3.1.1.2. IQ is the documented verification that the equipment or systems, as installed or
18727 modified, comply with the approved design, the manufacturer's recommendations
18728 and/or user requirements IQ should be performed on all critical facilities, systems and
18729 equipment. The IQ protocol should include, but not be limited to:

18730 7.3.1.1.2.1. verification that all facilities and equipment comply with the requirements of the
18731 purchase order;

18732 7.3.1.1.2.2. verification of CE-approval where required;

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18733 7.3.1.1.2.3. verification that the location and environmental conditions of the
18734 equipment/installation are correct according to the manufacturer's recommendations
18735 and internal specifications;

18736 7.3.1.1.2.4. verification that items are installed in accordance with internal specifications and
18737 identified correctly with the manufacturer;

18738 7.3.1.1.2.5. verification of serial numbers of all items/parts;

18739 7.3.1.1.2.6. verification that all parts of the equipment are free from defects;

18740 7.3.1.1.2.7. verification that the connections of electricity, water, steam, pressure, vacuum, etc.
18741 are functional and that their operating ranges are appropriate to the proper
18742 functioning of the installation;

18743 7.3.1.1.2.8. identification of the items that require calibration. Check for appropriate calibration
18744 certificates and programme and procedure for periodic calibration;

18745 7.3.1.1.2.9. verification of the existence of instructions for performing preventive maintenance.

18746 7.3.1.1.3. OQ is the documented verification that the equipment or systems, as installed or
18747 modified, perform as intended throughout the anticipated operating ranges) should
18748 follow IQ. The OQ protocol should include, but not be limited to the following:

18749 7.3.1.1.3.1. tests that have been developed from knowledge of processes, systems and equipment;

18750 7.3.1.1.3.2. tests to include a condition or a set of conditions encompassing upper and lower
18751 operating limits, sometimes referred to as 'worst case' conditions;

18752 7.3.1.1.3.3. identification of critical operating variables, tests performed, alarms, security devices
18753 and acceptance criteria;

18754 7.3.1.1.3.4. verification that the operation of various items of equipment / installation connected
18755 to the mains and put into operation is correct.

18756 7.3.1.1.4. The completion of a successful OQ should allow the finalisation of calibration,
18757 operating and cleaning procedures, operator training and preventative maintenance
18758 requirements. It should permit a formal 'release' of the facilities, systems and
18759 equipment.

18760 7.3.1.1.5. PQ is the documented verification that the equipment and ancillary systems, as
18761 connected together, can perform effectively and reproducibly based on the approved
18762 process method and specifications) should follow successful completion of IQ and OQ.
18763 Although PQ is described as a separate activity, it may in some cases be appropriate to
18764 perform it in conjunction with OQ, or concurrently with processing activities. The PQ
18765 protocol should include, but not be limited to, the following:

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18766 7.3.1.1.5.1. tests, using materials, qualified substitutes or simulated tissues and cells, that have
18767 been developed from knowledge of the process and the facilities, systems or
18768 equipment;

18769 7.3.1.1.5.2. tests to include a condition or set of conditions encompassing upper and lower
18770 operating limits;

18771 7.3.1.1.5.3. process description or reference to protocol development and / or conditioning to
18772 validate;

18773 7.3.1.1.5.4. list of equipment involved;

18774 7.3.1.1.5.5. critical parameters and operating ranges;

18775 7.3.1.1.5.6. reference of the procedures involved;

18776 7.3.1.1.5.7. description of the tests to be performed, or control variables, sample taking, time and
18777 reference method sampling and analytical methods;

18778 7.3.1.1.5.8. acceptance criteria.

18779 7.3.2. *Qualification of established (in-use) facilities, systems and equipment*

18780 7.3.2.1. Evidence should be available to support and verify the operating parameters and limits
18781 for the critical variables of the operating equipment.

18782 7.3.2.2. The calibration, cleaning, preventative maintenance, operating procedures and
18783 operator training procedures and records of the in-use facilities / systems / equipment
18784 should be documented.

18785 7.3.3. *Qualification of Clean Rooms*

18786 7.3.3.1. Clean rooms and laminar flow cabinets must be classified, re-qualified and monitored
18787 in accordance with EN ISO14644 and EU GMP Annex 1.The associated tests to be
18788 carried out for clean rooms should include at least:

18789 7.3.3.1.1. air change (renewal) rate per hour within one room: the speed and rate of renewals
18790 per hour according to specified will be checked;

18791 7.3.3.1.2. airflow visualisation test ;

18792 7.3.3.2. absolute filters integrity: the grade of sealing of the filters and the absence of leaks in
18793 the filter material will be checked;

18794 7.3.3.3. particle counting: the total count of airborne particles (viable or not) will be checked
18795 according to specifications;

18796 7.3.3.4. temperature / relative humidity: the temperature and relative humidity will be
18797 recorded during the test and will be checked according to specifications;

18798 7.3.3.5. differential pressure: the pressure differential between the different areas will be
18799 checked according to specifications;

18800 7.3.3.6. recovery test (normally tested for A and B classified clean rooms): the time required
18801 for a clean room to recover the specified classification after an out-of specifications
18802 will be checked;

18803 7.3.3.7. laminar flow velocities in laminar flow areas;

18804 7.3.3.8. HVAC system operations and alarms;

18805 7.3.3.9. electricity back-up systems. All these tests should be performed at least in an 'at rest'
18806 situation. Additionally, the particle counting test should be performed also in an 'in
18807 operation' situation.

18808 7.3.4. *Qualification of Laminar Flow Hoods*

18809 *7.3.4.1.* *The tests to be carried out for laminar flow hoods should include:*

18810 7.3.4.1.1. speed and uniformity of the air: the average speed meets the specified acceptance
18811 criteria and that there is uniformity will be checked;

18812 7.3.4.1.2. absolute filters integrity: the grade of sealing of the filters and the absence of leaks in
18813 the filter material will be checked;

18814 7.3.4.1.3. particle counting: the total count of airborne particles (viable or not) will be checked
18815 according to specifications;

18816 7.3.4.1.4. electronic test: all the operating controls will be checked (light, Ultra Violet (UV) light,
18817 fan) and alarms;

18818 7.3.4.1.5. Airflow visualisation (for biological safety cabinets). The test objective is to study the
18819 behaviour of air inside and outside the cabin with the help of a smoke generator. All
18820 these tests should be performed at least in an 'at rest' situation. Additionally, the
18821 particle counting test should be performed also in an 'in operation' situation.

18822 7.4. *Process validation*

18823 7.4.1. Facilities, systems and equipment to be used should have been qualified and analytical
18824 testing methods should be validated.

18825 7.4.2. Processes in use for some time should also be validated.

18826 7.4.3. Staff taking part in the validation work should have been appropriately trained.

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18827 7.4.4. Facilities, systems, equipment and processes should be periodically evaluated to verify
18828 that they are still operating in a valid manner.

18829 7.4.5. *Prospective validation*

18830 7.4.5.1. Process validation should normally be completed prior to the distribution of any tissue
18831 or cell (prospective validation).

18832 7.4.5.2. Prospective validation should include, but not be limited to the following:

18833 7.4.5.2.1. short description of the process;

18834 7.4.5.2.2. summary of the critical processing steps to be investigated;

18835 7.4.5.2.3. list of the equipment/facilities to be used (including measuring / monitoring /
18836 recording equipment) together with its calibration status;

18837 7.4.5.2.4. specifications for release of the processed tissues and cells;

18838 7.4.5.2.5. list of analytical methods, as appropriate;

18839 7.4.5.2.6. proposed in-process controls with acceptance criteria;

18840 7.4.5.2.7. additional testing to be carried out, with acceptance criteria and analytical validation,
18841 as appropriate;

18842 7.4.5.2.8. sampling plan;

18843 7.4.5.2.9. methods for recording and evaluating results;

18844 7.4.5.2.10. functions and responsibilities;

18845 7.4.5.2.11. proposed timetable.

18846 7.4.5.3. Using this defined process (including specified components) a series of batches of the
18847 final tissues or cells may be produced under routine conditions.

18848 7.4.5.4. The number of process runs carried out and observations made should be sufficient to
18849 allow the normal extent of variation and trends to be established and to provide
18850 sufficient data for evaluation. It is generally considered acceptable that three
18851 consecutive batches/runs within the finally agreed parameters would constitute a
18852 validation of the process.

18853 7.4.5.5. Batches, where applicable, made for process validation should be the same size as the
18854 routine scale batches.

18855 7.4.6. *Concurrent validation*

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18856 7.4.6.1. In exceptional circumstances it may be acceptable not to complete a validation
18857 program before routine processing starts and to validate a processes during routine
18858 processing (concurrent validation). The decision to carry out concurrent validation
18859 must be justified, documented and approved by authorised personnel.

18860 7.4.6.2. Documentation requirements for concurrent validation are the same as specified for
18861 prospective validation.

18862 7.4.7. *Retrospective validation*

18863 7.4.7.1. Retrospective validation is only acceptable for well-established processes. Validation
18864 of such processes should be based on historical data. The steps involved require the
18865 preparation of a specific protocol and the reporting of the results of the data review,
18866 leading to a conclusion and a recommendation

18867 7.4.7.2. Retrospective validation is not appropriate where there have been recent changes in
18868 relation to starting materials, the tissues or cells, operating procedures or equipment.

18869 7.4.7.3. The source of data for this validation should include, but not be limited to:

18870 7.4.7.3.1. batch processing and packaging records;

18871 7.4.7.3.2. process control charts;

18872 7.4.7.3.3. maintenance log books;

18873 7.4.7.3.4. records of personnel changes;

18874 7.4.7.3.5. process capability studies;

18875 7.4.7.3.6. processed tissues and cells data;

18876 7.4.7.3.7. storage stability results.

18877 7.4.7.4. Batches selected for retrospective validation should be representative of all batches
18878 made during the review period, including any batches that failed to meet
18879 specifications, and should be sufficient in number to demonstrate process consistency.

18880 7.4.7.5. Additional testing of retained samples may be needed to obtain the necessary amount
18881 or type of data to retrospectively validate the process.

18882 7.4.7.6. For retrospective validation, generally data from ten to thirty consecutive batches
18883 should be examined to assess process consistency, but fewer batches may be
18884 examined if justified.

18885 7.4.8. Process validation for aseptic processing in Grade A areas with Grade B background.

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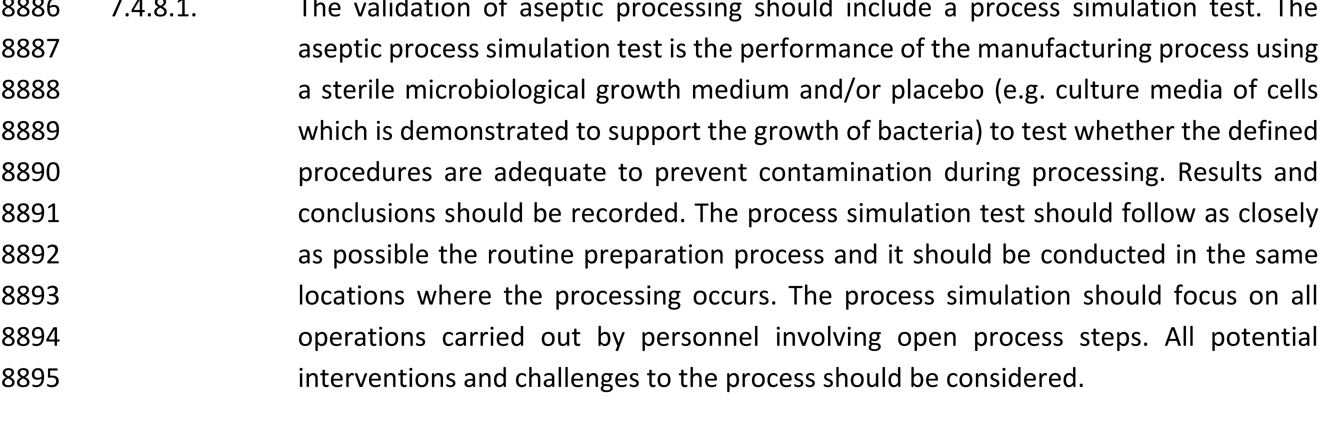

7.4.8.1. The validation of aseptic processing should include a process simulation test. The aseptic process simulation test is the performance of the manufacturing process using a sterile microbiological growth medium and/or placebo (e.g. culture media of cells which is demonstrated to support the growth of bacteria) to test whether the defined procedures are adequate to prevent contamination during processing. Results and conclusions should be recorded. The process simulation test should follow as closely as possible the routine preparation process and it should be conducted in the same locations where the processing occurs. The process simulation should focus on all operations carried out by personnel involving open process steps. All potential interventions and challenges to the process should be considered.

7.4.8.2. An appropriate simulated model using alternative materials may be acceptable provided that this is duly justified.

7.4.8.3. Alternative approaches may also be developed for steps that take a long time. The simulation of reduced times for certain activities (e.g. centrifugation, incubation) should be justified having regard to the risks. In some cases, it may also be acceptable to split the process into key stages which are simulated separately provided that the transitions between each stage are also evaluated. When a closed system is used for the processing of tissues and cells, the process simulation should focus on the steps related to the connections to the closed system.

7.4.8.4. In case of processing of various types of tissues and cells, consideration can be given to the matrix and/or bracketing approach. Under a bracketing approach, only samples on the extremes of certain design factors would undergo a full process simulation. This approach can be accepted if the handling of different tissues and cells is similar (same equipment and processing steps). Under a matrix approach, it may be possible to combine process simulations for different tissues and cells sharing similar processing steps, provided that the worst case is covered by the matrix approach. The use of bracketing and matrixing together should be duly justified.

7.4.8.5. Filled containers should be inverted to ensure the media/placebo touches all parts of the container/closure and should be incubated. The selection of the incubation duration and temperature should be justified and appropriate for the process being simulated and the selected media/placebo.

7.4.8.6. All contaminants from the filled containers should be identified. The results should be assessed, in particular in relation to the overall quality of the processes tissues and cells and the suitability of the preparation process. The target should be zero growth. Any growth detected should be investigated. If the growth detected is indicative of potential systemic failure, the potential impact on tissues and cells processed since the last successful media fill simulation test should be assessed and adequate corrective and preventive actions should be taken

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18928 7.4.8.7. Process simulation test to support initial validation should be performed with three
18929 consecutive satisfactory simulation tests per preparation process.
18930
18931 7.4.8.8. Process simulation (one run) should be repeated periodically to provide ongoing
18932 assurance of the ability of the process and the staff to ensuring aseptic manufacturing.
18933 The frequency should be determined based on a risk assessment but should generally
18934 not be lower than once every six months (for each preparation process).
18935
18936 7.4.8.9. In the case of infrequent processing (i.e. if the interval between the processing
18937 activities is more than six months), it is acceptable that the process simulation test is
18938 done just before the next scheduled processing activity, provided that the results of
18939 the process simulation test are available prior to the start of processing. Nevertheless,
18940 in cases of long periods of inactivity (i.e. over one year), the validation prior to restart
18941 of processing should be done with three runs.
18942
18943 7.4.8.10. When considering the frequency of the simulation test, the establishment is required
18944 to consider also the relevance of the simulation test for the training of personnel and
18945 their ability to operate in an aseptic environment.
18946
18947 7.4.8.11. A process simulation should also be conducted in cases when there is any significant
18948 change to the process (e.g. modification of HVAC system, equipment, etc). In this case,
18949 three runs are required.
18950
18951 7.5. *Cleaning and Disinfection Validation*

18952 7.5.1. Cleaning and disinfection validation should be performed in order to confirm the
18953 effectiveness of a cleaning or disinfection procedure.

18954 7.5.2. The rationale for selecting limits of carry-over of tissues and cells residues, cleaning
18955 agents and microbial contamination should be logically based on the materials
18956 involved. The limits should be achievable and verifiable.

18957 7.5.3. Residues of tissues and cells or cleansing agents should be checked based on risk
18958 assessment. Validated analytical methods having sensitivity to detect residues or
18959 contaminants should be used. The detection limit for each analytical method should
18960 be sufficiently sensitive to detect the established acceptable level of the residue or
18961 contaminant.

18962 7.5.4. Normally only cleaning or disinfection procedures for tissues and cells contact surfaces
18963 of the equipment need to be validated. Consideration should be given to noncontact
18964 parts.

18965 7.5.5. The intervals between use and cleaning or disinfection as well as cleaning or
18966 disinfection and reuse should be validated.

18967 7.5.6. Cleaning or disinfection intervals and methods should be determined.

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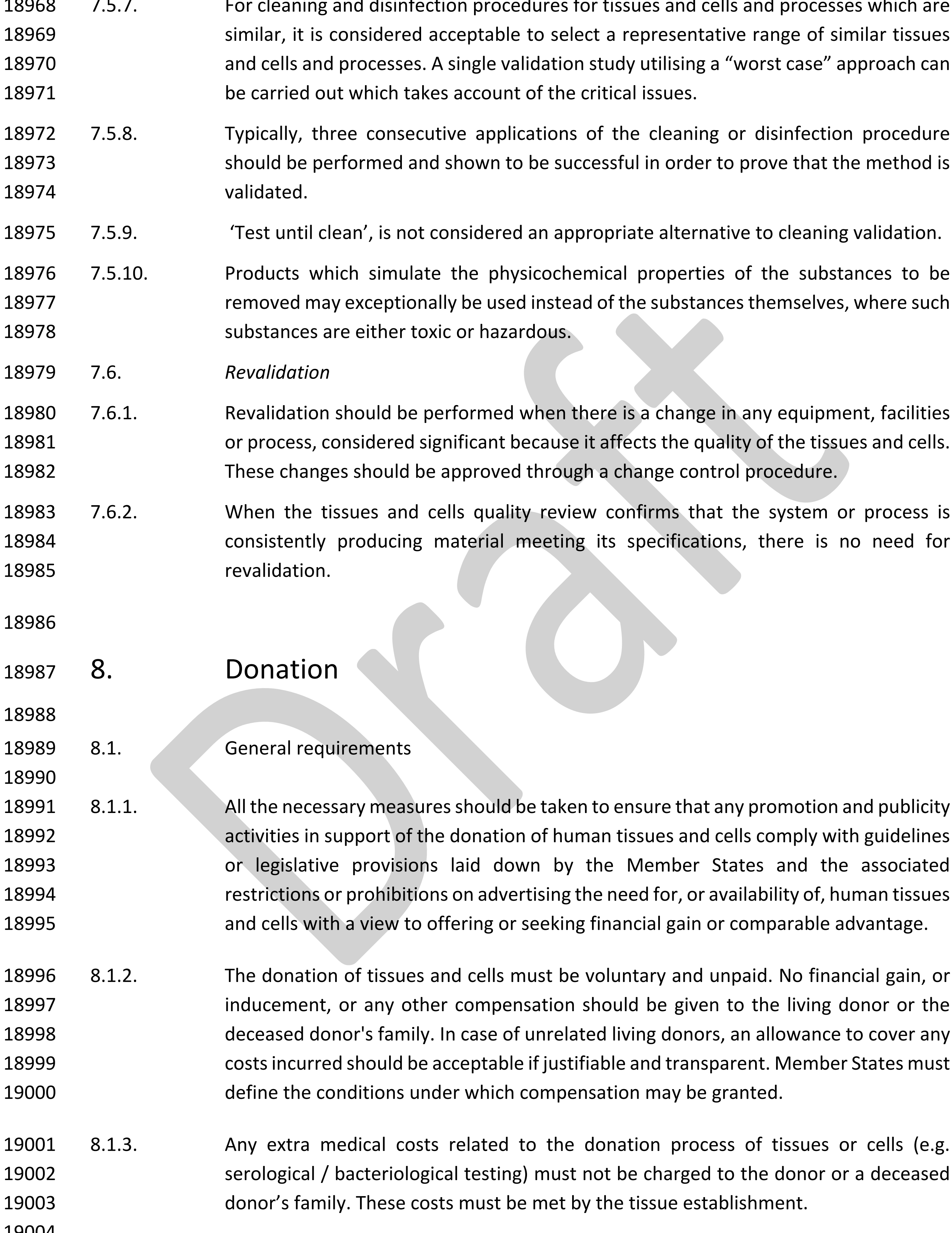
7.5.7. For cleaning and disinfection procedures for tissues and cells and processes which are similar, it is considered acceptable to select a representative range of similar tissues and cells and processes. A single validation study utilising a “worst case” approach can be carried out which takes account of the critical issues.

7.5.8. Typically, three consecutive applications of the cleaning or disinfection procedure should be performed and shown to be successful in order to prove that the method is validated.

7.5.9. ‘Test until clean’, is not considered an appropriate alternative to cleaning validation.

7.5.10. Products which simulate the physicochemical properties of the substances to be removed may exceptionally be used instead of the substances themselves, where such substances are either toxic or hazardous.



7.6. *Revalidation*

7.6.1. Revalidation should be performed when there is a change in any equipment, facilities or process, considered significant because it affects the quality of the tissues and cells. These changes should be approved through a change control procedure.

7.6.2. When the tissues and cells quality review confirms that the system or process is consistently producing material meeting its specifications, there is no need for revalidation.

8. Donation

8.1. General requirements

8.1.1. All the necessary measures should be taken to ensure that any promotion and publicity activities in support of the donation of human tissues and cells comply with guidelines or legislative provisions laid down by the Member States and the associated restrictions or prohibitions on advertising the need for, or availability of, human tissues and cells with a view to offering or seeking financial gain or comparable advantage.

8.1.2. The donation of tissues and cells must be voluntary and unpaid. No financial gain, or inducement, or any other compensation should be given to the living donor or the deceased donor's family. In case of unrelated living donors, an allowance to cover any costs incurred should be acceptable if justifiable and transparent. Member States must define the conditions under which compensation may be granted.

8.1.3. Any extra medical costs related to the donation process of tissues or cells (e.g. serological / bacteriological testing) must not be charged to the donor or a deceased donor’s family. These costs must be met by the tissue establishment.

19005 8.1.4. The activities related to tissue and cell procurement must be carried out in such a way
19006 as to ensure that donor evaluation and selection is carried out in accordance with the
19007 requirements referred to in Article 28(d) and (e).
19008 8.1.5. In the case of an autologous donation, the suitability criteria must be established in
19009 accordance with the requirements referred to in Article 28(d) of Directive 2004/23/EC.

19010 8.1.6. The results of the donor evaluation and testing procedures must be documented, and
19011 any major anomalies must be reported in accordance with the requirements referred
19012 to in the Annex of Directive 2004/23/EC.

19013 8.1.7. Tissue establishments must take all necessary measures to ensure that tissue and cell
19014 procurement, packaging and transportation complies with the requirements referred
19015 to in Article 28(b), (e) and (f) of Directive 2004/23/EC. The tests required for donors
19016 must be carried out by a laboratory accredited, designated, authorised or licensed by
19017 the health authority or authorities.

19018 8.1.8. Tissue establishments must implement a system for the identification of human tissues
19019 and cells, in order to ensure the traceability of all human tissues and cells.

19020 8.1.9. The procurement of human tissues or cells must be authorised only after all mandatory
19021 consent or authorisation requirements in force in the Member State concerned have
19022 been met.

19023 8.1.10. Tissue establishments must, in keeping with the national legislation, take all necessary
19024 measures to ensure that donors, their relatives or any persons granting authorisation
19025 on behalf of the donors are provided with all appropriate information as referred to in
19026 the Annex of Directive 2004/23/EC.

19027 8.1.11. Before the procurement of tissues and cells proceeds, an authorised person must
19028 confirm and record:
19029 8.1.11.1. that consent for the procurement has been obtained in accordance with Article 13 of
19030 Directive 2004/23/EC;
19031 8.1.11.2. how and by whom the donor has been reliably identified.
19032
19033 8.2. *Donor recruitment/ Donor referral*

19034 8.2.1. Procedures for the recruitment of living donors in an ethical manner and ensuring their
19035 safety and well-being and for the identification and referral of all potential deceased
19036 donors should be implemented and maintained in accordance with the applicable
19037 legislation.
19038
19039 8.2.2. Professionals involved in the recruitment of living donors, and in the identification and
19040 referral of deceased donors must be trained and appropriately qualified.

19041 8.2.3. In the case of both living and deceased donors, screening must be performed to
19042 exclude any contraindications to donate.

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19043

19044 8.2.4. A coding system, physical and documental and / or electronic, must be in place to
19045 guarantee traceability and biovigilance at all stages from donor screening until tissue
19046 and cell application (e.g. the donor is identified with a wrist band and/or different
19047 labels attached to the body). Both identification methods include the donor number.
19048 The donor code is applied to all tissues obtained after procurement.

19049 8.2.5. The coding system must be designed to relate all transplants of a certain donor to a
19050 unique donor number in order to guarantee traceability and biovigilance from donor
19051 screening until tissue transplantation.

19052 8.2.6. A potential donor should receive a donor identification number before any further
19053 procedures are started. All documental and/or electronic data that are collected from
19054 the donor, should state this number. All body materials (e.g. blood, tissue, fluid) that
19055 are procured from this donor, should refer to the donor number.

19056 8.2.7. The method of verifying the donor's identity should be described in an identification
19057 procedure. This procedure should be followed before starting the procurement and
19058 should enable the identity of the donor to be established beyond any doubt. The
19059 verification should be performed based on at least two independent factors like date
19060 of birth and name, or name and hospital patient number.

19061 8.2.8. The source of the donor's identity should be documented. For living donors this should
19062 include officially recognised means of identification such as identity cards, passports,
19063 etc. For deceased donors, the presence of toe tags, wrist bands or other confirmation
19064 of the deceased's identity should be noted. At least two independent forms of
19065 identification, such as name, date of birth, address, or hospital number, must be used
19066 to verify a deceased donor's identity.

19067 8.2.9. *Living donors*

19068

19069 8.2.9.1. Recruitment of donors must be voluntary and unpaid, and informed consent must be
19070 obtained in advance.

19071

19072 8.2.9.2. Recruitment of non-partner donors in MAR, whether performed by public health
19073 system or by private clinics (where allowed by national legislation), must be authorised
19074 by the health authority and the donation of reproductive material must strictly follow
19075 the same principles of voluntary and unpaid donation.

19076 8.2.9.3. Recruitment of persons not able to consent should never be done through public
19077 registries. In addition, in some countries, specific regulations restrict donation in these
19078 circumstances (e.g. some countries do not allow procurement of peripheral blood
19079 progenitor cells from minors and/or administration of growth factors).

19080 8.2.10. *Deceased donors*

19081

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19082 8.2.10.1. Identification and referral of deceased tissue donors must be in compliance with the
19083 national deceased donation programme.
19084
19085 8.2.10.2. A system to ensure that any deceased individual should be detected in an adequate
19086 period of time to perform an effective donation must be established between the
19087 procurement organisation and the corresponding tissue establishment or
19088 organisations responsible for human application.

19089 8.3. *Donor consent*
19090
19091 8.3.1. Procedures or protocols for expressing consent to donation, depending on the type of
19092 donor, the specific circumstances and the different legal systems for consent must be
19093 implemented and maintained.

19094 8.3.2. There should be an authorised person who confirms and records that consent for the
19095 procurement has been obtained in accordance with Article 13 of Directive 2004/23/EC
19096 and the legislation in place in the Member State.
19097
19098 8.3.3. Consent should be recorded and/or documented in the donor/patient's record.
19099
19100 8.3.3.1. Informed consent must be obtained for living donors. The informed consent must
19101 include an explanation, in understandable terms, of all the reasonable risk and
19102 potential harm, both for the donor and recipient, as well as all the tests to be
19103 performed.
19104
19105 8.3.3.2. Informed consent must be obtained from all donors for the use of their tissue and/or
19106 cell for specific purposes and for serological testing.

19107 8.3.3.3. The person in charge of the donation process must ensure that the donor has been
19108 properly informed of at least those aspects relating to the donation and procurement
19109 process outlined in point 8.4.5. Information must be given prior to the procurement.
19110
19111 8.3.3.4. The information must be given by a trained and appropriately qualified person able to
19112 transmit it in an appropriate and clear manner, using terms that are easily understood
19113 by the donor.

19114 8.3.3.5. The information must cover: the purpose and nature of the procurement, its
19115 consequences and risks; analytical tests, if they are performed; recording and
19116 protection of donor data, medical confidentiality; therapeutic purpose and potential
19117 benefits and information on the applicable safeguards intended to protect the donor.

19118 8.3.3.6. The donor must be informed that he/she has the right to receive the confirmed results
19119 of the analytical tests, clearly explained.
19120

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8.3.3.7. Information must be given on the necessity for requiring the applicable mandatory consent, certification and authorisation in order that the tissue and/or cell procurement can be carried out.

8.3.4. Tissue or cell procurement must not be carried out on a person who does not have the capacity to consent, other than

8.3.4.1. In case of minors donors or donor with no legal capacity the consent must be obtained from parents or legal representative, provided the following conditions are met:

8.3.4.1.1. there is no compatible donor available who has the capacity to consent;

8.3.4.1.2. the recipient is a brother or sister of the donor;

8.3.4.1.3. the donation has the potential to be life-saving for the recipient;

8.3.4.1.4. the authorisation of his or her representative or an authority or a person or body provided for by law has been given specifically and in writing and with the approval of the competent body;

8.3.4.1.5. the potential donor concerned does not object.

8.3.5. In the case of discarded tissue or surgical by-product, the patient must be made aware that he or she can express any intention as to how he or she desires such surgical residues to be dealt with or else the surgical residues should be handled by the healthcare institutions as it deems fit.

8.3.5.1. All information must be given and all necessary consents and authorisations must be obtained in accordance with the legislation in force in Member States.

8.3.5.2. The confirmed results of the donor's evaluation must be communicated and clearly explained to the relevant persons in accordance with the legislation in Member States.

8.3.5.3. In case a legal process applies, judicial consent must be obtained according to local regulations before starting any procurement activities. When judicial authorization is needed due to an unknown cause of death, the transplant coordinator should be the person responsible to ask consent from the judge on call and in charge of the investigation. Procurement should only be carried out if it does not affect the judicial autopsy.

8.3.6. The request for donation must be explained in understandable terms by a health care professional familiar with the donation process.

8.3.6.1. The discussion about consent should be conducted in a suitable environment. The person who requests the consent of the donor should have received specific training for this purpose.

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19162 8.3.6.2. The use of tissue from deceased donor must be in accordance with relevant national
19163 and international legislation.
19164
19165 8.4. *Donor selection and evaluation*
19166
19167 8.4.1. Procedures for donor selection and evaluation should be implemented and
19168 maintained. They should take place before each procurement and comply with
19169 requirements referred to as above.
19170
19171 8.4.2. Selection criteria for donors are based on an analysis of the risks related to the
19172 application of the specific cells/tissues. Indicators of these risks must be identified by
19173 review of the medical and behavioural history, biological testing, physical examination
19174 (for living donors) or post-mortem examination (for deceased donors) and any other
19175 appropriate investigation.
19176
19177 8.4.3. The TE or procurement organization must ensure that donors comply with the
19178 selection criteria set out in Directive 2006/17/EC:
19179
19180 8.4.3.1. Annex I for donors of tissues and cells, except donors of reproductive cells;
19181
19182 8.4.3.2. Annex III for donors of reproductive cells.
19183
19184 8.4.4. The health TE or procurement organization must ensure that:
19185
19186 8.4.4.1. donors of tissues and cells, except donors of reproductive cells, undergo the biological
19187 tests set out in point 1 of Annex II of Directive 2006/17/EC;
19188
19189 8.4.4.2. the tests referred to in point 8.4.4.1. are carried out in compliance with the general
19190 requirements set out in point 2 of Annex II of Directive 2006/17/EC.
19191
19192 8.4.4.3. donors of reproductive cells undergo the biological tests set out in points 1, 2 and 3 of
19193 Annex III of Directive 2006/17/EC;
19194
19195 8.4.4.4. the tests referred to in point 8.4.4.3 above are carried out in compliance with the
19196 general requirements set out in point 4 of Annex III of Directive 2006/17/EC.
19197
19198 8.4.5. In the case of living donors, the health professional responsible for obtaining the health
19199 and social history must ensure that the donor has:
19200
19201 8.4.5.1. understood the information provided;
19202
19203 8.4.5.2. had an opportunity to ask questions and been provided with satisfactory responses;
19204
19205 8.4.5.3. confirmed that all the information provided is true to the best of his/her knowledge.

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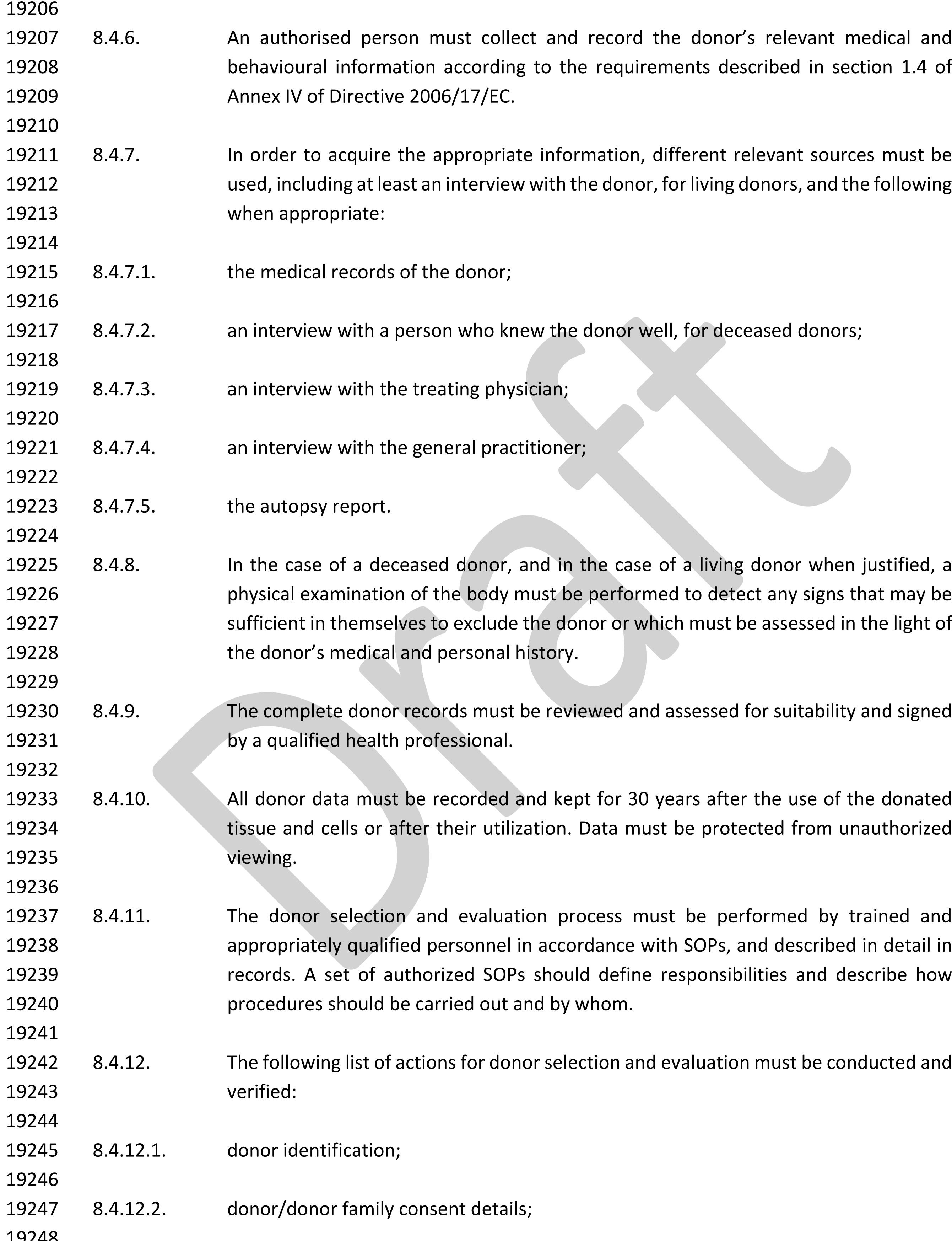

8.4.6. An authorised person must collect and record the donor's relevant medical and behavioural information according to the requirements described in section 1.4 of Annex IV of Directive 2006/17/EC.

8.4.7. In order to acquire the appropriate information, different relevant sources must be used, including at least an interview with the donor, for living donors, and the following when appropriate:

8.4.7.1. the medical records of the donor;

8.4.7.2. an interview with a person who knew the donor well, for deceased donors;

8.4.7.3. an interview with the treating physician;

8.4.7.4. an interview with the general practitioner;

8.4.7.5. the autopsy report.

8.4.8. In the case of a deceased donor, and in the case of a living donor when justified, a physical examination of the body must be performed to detect any signs that may be sufficient in themselves to exclude the donor or which must be assessed in the light of the donor's medical and personal history.

8.4.9. The complete donor records must be reviewed and assessed for suitability and signed by a qualified health professional.

8.4.10. All donor data must be recorded and kept for 30 years after the use of the donated tissue and cells or after their utilization. Data must be protected from unauthorized viewing.

8.4.11. The donor selection and evaluation process must be performed by trained and appropriately qualified personnel in accordance with SOPs, and described in detail in records. A set of authorized SOPs should define responsibilities and describe how procedures should be carried out and by whom.

8.4.12. The following list of actions for donor selection and evaluation must be conducted and verified:

8.4.12.1. donor identification;

8.4.12.2. donor/donor family consent details;

8.4.12.3. donor's medical history (including genetic disease, a family history of disease, exclusion criteria, additional exclusion criteria for deceased child donors);

8.4.12.4. donor's social history (including personal, travel, behavioural, risk assessment);

8.4.12.5. donor's physical examination (including exclusion signs);

8.4.12.6. donor's psychological examination (living haematopoietic progenitor cells and medically assisted reproduction donors);

8.4.12.7. blood sample procurement;

8.4.12.8. evaluation of test results for markers of transmissible disease;

8.4.12.9. final decision-making about eligibility.

8.4.13. In the case of living donors, face-to-face interview must be conducted, during which a standardized questionnaire should be completed. The interviews should be done, documented and signed by a trained and appropriately qualified person. For deceased donors, alternative sources of information should be used.

8.4.14. *Living donors*

8.4.14.1. *Autologous living donor*

8.4.14.1.1. If the removed tissues and cells are to be stored or cultured, the same minimum set of biological testing requirements must apply as for an allogeneic living donor.

8.4.14.1.2. Positive test results will not necessarily prevent the tissues or cells or any product derived from them being stored, processed and reimplanted, if appropriate isolated storage facilities are available to ensure no risk of cross contamination with other grafts and/or no risk of contamination with adventitious agents and/or mix ups.

8.4.14.2. Allogeneic living donor (except donors of reproductive cells)

8.4.14.2.1. Allogeneic living donors must be selected on the basis of their health and medical history, healthcare professional with the donor, in compliance with point 8.4.14.2.3. This assessment must include relevant factors that may assist in identifying and screening out persons whose donation could present a health risk to others, such as the possibility of transmitting diseases or health risks to themselves.

8.4.14.2.2. For any donation, the procurement process must not interfere with or compromise the health or care of the donor. In the case of cord blood or amniotic membrane donation, this applies to both mother and baby.

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8.4.14.2.3. Selection criteria for allogeneic living donors must be established and documented by the tissue establishment (and the transplanting clinician in the case of direct distribution to the recipient), based on the specific tissue or cells to be donated, together with the donor's physical status and medical and behavioural history and the results of clinical investigations and laboratory tests establishing the donor's state of health.

8.4.14.2.4. The same exclusion criteria must be applied as for deceased donors with the exception of the concerning to the unknown cause of death. Depending on the tissue or cell to be donated, other specific exclusion criteria may need to be added, such as:

8.4.14.2.4.1. in the case of haematopoietic progenitor cells, the potential for transmission of inherited conditions.

8.4.14.3. *Donor of reproductive cells*

8.4.14.3.1. Donor selection criteria and laboratory testing do not need to be applied in the case of partner donation of reproductive cells for direct use.

8.4.14.3.2. Reproductive cells that are processed and/or stored and reproductive cells that will result in the cryopreservation of embryos must meet the following criteria:

8.4.14.3.2.1. the clinician responsible for the donor must determine and document, based on the patient's medical history and therapeutic indications, the justification for the donation and its safety for the recipient and any child(ren) that might result;

8.4.14.3.2.2. the biological tests must be carried out to assess the risk of cross-contamination in compliance with the general requirements set out in point 2 of Annex III of Directive 2006/17/EC;

8.4.14.3.2.3. positive results will not necessarily prevent partner donation in accordance with national rules.

8.4.14.3.3. Donor selection criteria and laboratory testing in the case of donations of reproductive cells other than by partners must be applied in accordance with Annex III, point 3 of Directive 2006/17/EC.

8.4.14.3.3.1. Donors must be selected on the basis of their age, health and medical history, provided on a questionnaire and through a personal interview performed by a qualified and trained healthcare professional.

8.4.14.3.3.2. This assessment must include relevant factors that may assist in identifying and screening out persons whose donation could present a health risk to others, such as the possibility of transmitting diseases (such as sexually transmitted infections), or

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19337 health risks to themselves (e.g. superovulation, sedation or the risks associated with
19338 the egg procurement procedure or the psychological consequences of being a donor).
19339
19340 8.4.14.3.3.3. Complete information on the associated risk and on the measures undertaken for its
19341 mitigation must be communicated and clearly explained to the recipient.
19342
19343 8.4.15. *Deceased donor*
19344
19345 8.4.15.1. National and local requirements for confirmation of death must be complied with
19346 before tissue procurement began.
19347
19348 8.4.15.2. In the case of deceased donors, the cause, time and circumstances of death must be
19349 verified and recorded. The sources of information used for deceased donors must be
19350 reviewed. Transferring information from donation records to a new document should
19351 be carried out by trained and appropriately qualified staff from the tissue
19352 establishment.
19353
19354 8.4.15.3. A donor age criteria should be established, documented and recorded.
19355
19356 8.4.15.4. The person responsible for confirming the donor's identity must do this by means of
19357 at least three factors, such as age, race, date of birth, or medical history number.

19358 8.4.15.5. General criteria for exclusion

19359 8.4.15.5.1. Cause of death unknown, unless autopsy provides information on the cause of death
19360 after procurement and none of the general criteria for exclusion set out in the present
19361 section applies.
19362
19363 8.4.15.5.2. History of a disease of unknown aetiology.
19364
19365 8.4.15.5.3. Presence, or previous history, of malignant disease, except for primary basal cell
19366 carcinoma, carcinoma in situ of the uterine cervix, and some primary tumours of the
19367 central nervous system that have to be evaluated according to scientific evidence and
19368 must be considered as part of the risk assessment in decision making. Donors with
19369 malignant diseases can be evaluated and considered for cornea donation, except for
19370 those with retinoblastoma, haematological neoplasm, and malignant tumours of the
19371 anterior segment of the eye. Malignant melanoma with known metastatic disease also
19372 excludes use of ocular tissue, including avascular cornea. Any vascularised ocular
19373 tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered
19374 by this exclusion and should be evaluated as discussed above.
19375
19376 8.4.15.5.4. Risk of transmission of diseases caused by prions. This risk applies, for example, to:
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19378 8.4.15.5.4.1. people diagnosed with Creutzfeldt–Jakob disease, variant Creutzfeldt-Jacob disease,
19379 or other prion disease or having a family history of non-iatrogenic Creutzfeldt-Jakob
19380 disease;
19381
19382 8.4.15.5.4.2. people with a history of rapid progressive dementia or degenerative neurological
19383 disease, including those of unknown origin;
19384
19385 8.4.15.5.4.3. recipients of hormones derived from the human pituitary gland (such as growth
19386 hormones) and recipients of grafts of cornea, sclera and dura mater, and persons that
19387 have undergone undocumented neurosurgery (where dura mater may have been
19388 used).

19389 8.4.15.5.5. Systemic infection which is not controlled at the time of donation, including bacterial
19390 diseases, systemic viral, fungal or parasitic infections, or significant local infection in
19391 the tissues and cells to be donated. Donors with bacterial septicaemia may be
19392 evaluated and considered for eye donation but only where the corneas are to be
19393 stored by organ culture to allow detection of any bacterial contamination of the tissue.
19394
19395 8.4.15.5.6. History, clinical evidence, or laboratory evidence of HIV, acute or chronic hepatitis B
19396 (HBV) (except in the case of persons with a proven immune status), hepatitis C (HCV)
19397 and HTLV I/II, transmission risk or evidence of risk factors for these infections.

19398 8.4.15.5.7. History of chronic, systemic autoimmune disease that could have a detrimental effect
19399 on the quality of the tissue to be retrieved.
19400
19401 8.4.15.5.8. Indications that test results of donor blood samples will be invalid due to:
19402
19403 8.4.15.5.8.1. the occurrence of haemodilution, according to the specifications in Annex II, section 2
19404 of Directive 2006/17/EC, where a pre-transfusion sample is not available;
19405
19406 8.4.15.5.8.2. Evidence of any other risk factors for transmissible diseases on the basis of a risk
19407 assessment, taking into consideration donor travel and exposure history and local
19408 infectious disease prevalence;
19409
19410 8.4.15.5.8.3. Presence on the donor's body of physical signs implying a risk of transmissible
19411 disease(s) as described in Annex IV, point 1.2.3 of Directive 2006/17/EC;
19412
19413 8.4.15.5.8.4. Ingestion of, or exposure to, a substance (such as cyanide, lead, mercury, gold) that
19414 may be transmitted to recipients in a dose that could endanger their health;
19415
19416 8.4.15.5.8.5. Recent history of vaccination with a live attenuated virus where a risk of transmission
19417 is considered to exist;
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19419 8.4.15.5.8.6. History of xenotransplantation that involves the transplantation, implantation or infusion into a human recipient of live xenogeneic cells, tissues or organs or of human bodily fluids, cells, tissues or organs that have had ex vivo contact with live xenogeneic materials (unless justified on the basis of a documented risk assessment).

19424 8.4.15.6. *Additional exclusion criteria for deceased child donors*

19426 8.4.15.6.1. Any children born from mothers with HIV infection or that meet any of the general exclusion criteria must be excluded as donors until the risk of transmission of infection can be definitely ruled out.

19430 8.4.15.6.1.1. Children aged less than 18 months born from mothers with HIV, hepatitis B, hepatitis C or HTLV infection, or at risk of such infection, and who have been breastfed by their mothers during the previous 12 months, cannot be considered as donors regardless of the results of the analytical tests.

19435 8.4.15.6.1.2. Children of mothers with HIV, hepatitis B, hepatitis C or HTLV infection, or at risk of such infection, and who have not been breastfed by their mothers during the previous 12 months and for whom analytical tests, physical examinations, and reviews of medical records do not provide evidence of HIV, hepatitis B, hepatitis C or HTLV infection, can be accepted as donors.

19441 8.4.16. Procedures should be in place to ensure that abnormal finding arising from the donor selection and evaluation process are properly reviewed by a qualified health professional ant that appropriate action is taken. The reason of rejection of a donor should be recorded.

9. Donor Testing

19447 9.1. Testing of donations for infectious markers and agents is a key factor in ensuring that the risk of disease transmission is minimised and that tissues and cells are suitable for their intended purpose.

19450 9.2. Each donation must be tested in conformity with the requirements laid down in EU Directives, especially in Annex II and III Directive 2006/17 EC.

19452 9.3. The tests must be carried out by a qualified laboratory, authorised as a testing centre by the health authority, using EC-marked testing kits where appropriate. The type of test used must be validated for the purpose in accordance with current scientific knowledge.

19456 9.4. Additional donor testing for other markers or agents may be required, taking into account the epidemiological profile in any given region or country.

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19458 19459 19460 9.5. The tests must be carried out on the donor's serum or plasma; they must not be performed on other fluids or secretions unless specifically justified clinically using a validated test for such a fluid.

19461 19462 19463 9.6. Tissue establishments may accept tissues and cells from donors with haemodilution of more than 50 % only if the testing procedures used are validated for such diluted plasma or if a pre-transfusion sample is available.

19464 9.7. *Donation samples*

19465 9.7.1. Deceased donors

19466 19467 9.7.1.1. In the case of a deceased donor, blood samples must have been obtained just prior to death or, if not possible as soon as possible after death (within 24 hours after death).

19468 9.7.2. *Living donors*

19469 19470 9.7.2.1. In the case of HPC donors, blood samples must be taken for testing within 30 days prior to donation.

19471 19472 *9.7.2.2. In the case of non-haematopoietic progenitor cells and non-reproductive cells living donors:*

19473 19474 9.7.2.2.1. where tissues and cells are going to be stored less than 180 days - blood samples must be obtained at the time of donation or, if not possible, within 7 days post donation.

19475 19476 19477 9.7.2.2.2. where tissues and cells are going to be stored 180 day or longer - blood samples can be taken up to 30 days prior to and 7 days post donation and repeat sampling and testing is required after an interval of 180 days.

19478 9.7.2.2.3. Repeat blood sampling and testing is not required after an interval of 180 days if:

19479 19480 19481 9.7.2.2.3.1. the blood sample, taken at the time of procurement or within 7 days after procurement, is additionally tested by the nucleic acid amplification technique (NAT) for HIV, HBV and HCV;

19482 19483 9.7.2.2.3.2. the processing includes an inactivation step that has been validated for the HIV, HBV and HCV;

19484 19485 9.7.2.3. In the case of neonatal donors, tests can be carried out on the donor's mother to avoid medically unnecessary procedures upon the infant.

19486 9.7.2.4. *In the case of reproductive non-partner donors:*

19487 9.7.2.4.1. All serum samples must be obtained at the time of donation

19488 9.7.2.4.2. Sperm donations must be quarantined for ≥ 180 days after the last procurement, after

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19489
19490 which repeat testing is required. Quarantine is not necessary if at each donation serology testing is combined with NAT for HIV, HBV and HCV.

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19495 9.7.2.4.3. The same testing approach must be used for oocyte donors, allowing for the safe use of cryopreserved oocytes (after quarantine and re-testing after 180 days) or fresh oocytes (if NAT is done at the time of donation). Oocyte donation may be considered as starting at the first day of stimulation, and the sample for testing may be taken at that time.

19496 9.7.2.5 *In the case of reproductive partner donors:*

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19500 9.7.2.5.1 Blood samples must be taken before the first donation and this must be done ≤ 3 months before the first donation. For further partner donations, additional blood samples must be obtained according to national legislation, but ≤ 24 months from the previous sampling.

19501
19502 9.8. The procedure used for the labelling of laboratory samples with donation numbers must be designed to avoid any risk of identification error and mix-up

19503
19504 9.9. Upon receipt of samples at the laboratory, positive identification of the samples received against those expected should be carried out.

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19506 9.10. Laboratory personnel must be thoroughly instructed, trained and competent to operate the test system.

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19509 9.11. Each step of the handling and processing of samples should be described, as should the conditions of pre-analytical treatment of specimens (e.g. centrifugation), storage and transportation (duration, temperature, type of container, storage after testing).

19510
19511 9.12. There must be data confirming the suitability of any laboratory reagents used in testing of donor samples.

19512 9.13. All laboratory testing procedures must be validated before use.

19513
19514
19515 9.14. Screening algorithms should be defined precisely in writing (i.e. Standard Operating Procedures) to deal with initially reactive specimens, and to resolve discrepancies in results after re-testing.

19516
19517 9.15. If additional NAT testing is performed, a thoroughly validated system of labelling/identification of samples should be in place.

19518
19519 9.16. There must be a reliable process in place for transcribing, collating and interpreting results.

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19521 9.17. There must be clearly defined procedures to resolve discrepant results. Appropriate confirmatory testing must take place. In the case of confirmed positive results,

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19522
19523 appropriate donor management must take place, including the provision of information to the donor and follow-up procedures.

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19526 9.18. The quality of the laboratory testing must be assessed regularly by participation in a formal system of proficiency testing, such as an external quality-assurance programme.

19527

19528 # 10. Procurement

19529 10.1. *General requirements*

19530 10.1.1. Procurement activities must be authorised by the appropriate health authority.

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19533 10.1.2. The procurement of human tissues or cells must be authorised only after all mandatory consent or authorisation requirements in force in the Member State concerned have been met.

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19535 10.1.3. Procurement of human tissues or cells must take place only after donor consent/authorisation requirements have been satisfied.

19536 10.2. *Procurement procedures*

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19539
19540 10.2.1. Procedures must be authorized and appropriate for the type of donor and the type of tissue or cells procured and must be standardized. The procurement procedures must be appropriate for the type of donor and the type of tissue/cells donated. There must be procedures in place to protect the safety of the living donor.

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19542
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19544 10.2.2. The procurement procedures must protect those properties of the tissue/cells that are required for their ultimate clinical use, and at the same time minimise the risk of microbiological contamination during the process, particularly when tissues and cells cannot subsequently be sterilised.

19545
19546 10.2.3. Policies and procedures must be in place to minimise the risk of tissue or cell contamination by staff who might be infected with transmissible diseases.

19547
19548 10.2.4. The sequence in which the various tissues are procured must be well defined to assure the quality of each type of tissue.

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19550 10.2.5. If deceased donation occurs after organ donation, sterility should be ensured throughout the whole procedure, including during organ procurement.

19551
19552 10.2.6. Selection of the use of suboptimal conditions must be supported by written justification and be authorized by the relevant Health Authority.

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19553 19554 10.2.7. Sample cultures of the tissues or cells procured should be taken and an appropriately validated culture method must be used.

19555 19556 10.2.8. Time limits for procurement should be validated by quality assessments and tests for microbiological contamination.

19557 19558 19559 10.2.9. Once the tissue is procured and until it arrives at the tissue establishment, critical variables related to maintaining the quality of the tissues or cells (e.g., temperature, sterile packaging) must be controlled and recorded.

19560 19561 19562 10.2.10. Once the tissues and cells have been retrieved from a deceased donor body, the donor body must be reconstructed so that it is as similar as possible to its original anatomical appearance.

19563 19564 19565 10.2.11. Where appropriate, the staff and equipment necessary for body reconstruction of deceased donors must be provided. Such reconstruction must be completed effectively.

19566 10.3. *Procurement report*

19567 19568 10.3.1. The organisation performing the procurement must produce a procurement report, which is passed on to the tissue establishment. This report must contain at least:

19569 19570 10.3.1.1. the identification, name and address of the tissue establishment to receive the cells/tissues;

19571 10.3.1.2. donor identification data (including how and by whom the donor was identified);

19572 19573 10.3.1.3. description and identification of procured tissues and cells (including samples for testing);

19574 19575 10.3.1.4. identification of the person who is responsible for the procurement session, including signing;

19576 19577 19578 19579 10.3.1.5. date, time (where relevant, start and end) and location of procurement and procedure (SOP) used, including any incidents that occurred; where relevant, environmental conditions at the procurement facility (description of the physical area where procurement took place);

19580 19581 10.3.1.6. for deceased donors, conditions under which the cadaver is kept: refrigerated (or not), time of start and end of refrigeration;

19582 10.3.1.7. ID/batch numbers of reagents and transport solutions used.

19583 10.3.2. The report must also contain the date and time of death where possible.

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19584 19585 10.3.3. Where sperm is procured at home, the procurement report must state this and must contain only:

19586 10.3.3.1. the name and address of the tissue establishment to receive the cells/tissues;

19587 10.3.3.2. the donor identification.

19588 10.3.4. The date and time of procurement may be included, where possible.

19589 19590 10.4. Following procurement, all recovered tissues and cells must be packaged and labelled as described in section 12 Packaging, coding and labelling.

19591

19592 11. Processing

19593 11.1. *General requirements*

19594 19595 19596 19597 19598 11.1.1. Each tissue and cell preparation process must be authorised by the health authority after evaluation of the donor selection criteria and procurement procedures, the protocols for each step of the process, the quality management criteria, and the final quantitative and qualitative criteria for cells and tissues. This evaluation must comply at least with the requirements set out in Directive 2006/86 Annex II.

19599 19600 19601 19602 11.1.2. Tissue establishments are responsible for the determination of suitability of the received tissues/cells for processing and for the quality and safety assessment of the processed tissue/cell products before distribution. Decisions regarding suitability should be made by a person who is appropriately qualified.

19603 11.2. *Reception of the tissues/cells at the tissue establishment*

19604 19605 19606 19607 19608 11.2.1. Each tissue establishment must ensure that the tissue and cells received are quarantined until they, along with the associated documentation, have been inspected or otherwise verified as conforming to requirements. The review of relevant donor/procurement information and thus acceptance of the donation needs to be carried out by specified/authorised persons.

19609 19610 19611 11.2.2. Tissue and cells must be held in quarantine until such time as the requirements relating to donor testing and information have been met in accordance with Directive 2004/23/EC Article 15.

19612 19613 19614 Received tissues and cells awaiting final test results, or subject to additional testing, or confirmatory medical assessment, must be placed under quarantine until such test results or medical data becomes available.

19615 19616 11.2.3. When the procured tissues/cells arrive at the tissue establishment, there must be documented verification that the consignment, including the transport conditions,

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19617 19618 19619 packaging, labelling and associated documentation and samples, meet the requirements of Annex IV of Directive 2006/17/EC and the specifications of the receiving establishment.

19620–19627 11.2.4. Each tissue establishment must have a documented policy and specifications against which each consignment of tissues and cells, including samples, are verified. These must include the technical requirements and other criteria considered by the tissue establishment to be essential for the maintenance of acceptable quality. The tissue establishment must have documented procedures for the management and segregation of non-conforming consignments, or those with incomplete test results, to ensure that there is no risk of contamination of other tissues and cells being processed, preserved or stored.

19628–19631 11.2.5. Tissue establishments must ensure that all donations of human tissues and cells are subjected to tests in accordance with the requirements referred to Directive 2004/23/EC Article 28(e) and that the selection and acceptance of tissues and cells comply with the requirements referred to in Directive 2004/23/EC Article 28(f).

19632–19634 11.2.6. Tissue establishments must ensure that human tissue and cells and associated documentation comply with the requirements referred to in Directive 2004/23/EC Article 28(f).

19635–19637 11.2.7. Tissue establishments must verify and record the fact that the packaging of human tissue and cells received complies with the requirements referred to in Article 28(f). All tissues and cells that do not comply with those provisions must be discarded.

19638–19639 11.2.8. The procurement report and shipping record (if the donation was transported by a third party) should be crosschecked with the contents of the package.

19640 11.2.9. The acceptance or rejection of received tissues or cells must be documented.

19641–19642 11.2.10. The data that must be registered at the tissue establishment (except for donors of reproductive cells intended for partner donation) include:

19643–19646 11.2.10.1. consent/authorisation; including the purpose(s) for which the tissues and cells may be used (i.e. therapeutic or research, or both therapeutic use and research) and any specific instructions for disposal if the tissue or cells are not used for the purpose for which consent was obtained;

19647–19648 11.2.10.2. all required records relating to the procurement and the taking of the donor history, as described in the donor documentation section;

19649–19650 11.2.10.3. results of physical examination, of laboratory tests and of other tests (such as the autopsy report, if used in accordance with Directive 2006/17/EC Annex IV point 1.2.2.);

19651 11.2.10.4. for allogeneic donors, a properly documented review of the complete donor
19652 evaluation against the selection criteria by an authorised trained and qualified person;

19653 11.2.10.5. in the case of cell cultures intended for autologous use, documentation of the
19654 possibility of medicinal allergies (such as to antibiotics) of the recipient.

19655 11.2.11. In the case of reproductive cells intended for partner donation, the data to be
19656 registered at the tissue establishment include:

19657 11.2.11.1. consent; including the purpose(s) for which the tissues and cells may be used (such as
19658 reproductive only and/or for research) and any specific instructions for disposal if the
19659 tissue or cells are not used for the purpose for which consent was obtained;

19660 11.2.11.2. partner identification;

19661 11.2.11.3. place of procurement;

19662 11.2.11.4. tissues and cells obtained and relevant characteristics.

19663 11.3. *Processing*

19664 11.3.1. Tissue establishments must include in their standard operating procedures all
19665 processes that affect quality and safety and must ensure that they are carried out
19666 under controlled conditions. Tissue establishments must ensure that the equipment
19667 used, the working environment and process design, validation and control conditions
19668 are in compliance with the requirements referred to in Directive 2004/23/EC Article
19669 28(h).

19670 11.3.2. Any modifications to the processes used in the preparation of tissues and cells must
19671 also meet the criteria laid down in the above paragraph.

19672 Any substantial modification to the processes and parameters in reference to tissue
19673 and cell processing should be reported to the national health authority and if required,
19674 authorized by the authority prior to its commencement.

19675 11.3.3. The procedures must be documented in SOPs which must conform to the validated
19676 method and to the standards laid down in this Directive, accordingly with Directive
19677 2006/86/ Annex I(E), points 1 to 4.

19678 11.3.4. It must be ensured that all processes are conducted in accordance with the approved
19679 SOPs.

19680 11.3.5. The critical processing procedures must be validated and must not render the tissues
19681 or cells clinically ineffective or harmful to the recipient. This validation may be based
19682 on studies performed by the establishment itself, or on data from published studies
19683 or, for well established processing procedures, by retrospective evaluation of the
19684 clinical results for tissues supplied by the establishment. Processing methods should

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be designed to ensure the safety and biological functionality of processed tissues and cells.

11.3.6. If validation is based on retrospective evaluation of the clinical results for tissues or cells supplied by the establishment, data should be collected and analysed that include the number of tissues or cells implanted following processing by the method under consideration, and the time period (start and end dates/times) during which these implantations occurred.

11.3.7. It should be demonstrated that the validated process can be carried out consistently and effectively in the tissue establishment environment by the staff.

11.3.8. If processing is carried out according to GMP, the processing validation must be done according to GMP guidelines.

11.3.9. If physicochemical methods are to be applied, these procedures must be adapted to the type of tissue or cell and should be validated.

11.3.10. The processing procedures must undergo regular critical evaluation to ensure that they continue to achieve the intended results.

11.3.11. Before implementing any significant change in processing, the modified process must be validated and documented.

11.3.12. Tissues or cells from different donors should not be pooled during processing unless this is the only way in which clinical efficacy can be achieved on an individual patient basis. Traceability must be fully ensured if pooling of different tissues and cells from two or more donors during processing is performed

11.3.13. Pooled tissues or cells should be treated as a single batch while ensuring that the original donations are fully traceable.

11.3.14. In case of pooling during the process, a risk evaluation should be in place to preclude cross-contamination.

11.3.15. If the process includes a sterilisation or viral-inactivation step, process-specific validation studies should be completed to demonstrate the log reduction achieved by the process.

11.3.16. Where a microbial inactivation procedure is applied to the tissue or cells, it must be specified, documented, and validated.

11.3.17. In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during processing or storage, with tissues or cells from another donor, unless they are intentionally pooled.

11.3.18. A separate set of clean, sterile instruments should be used for each donor.

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19719 11.3.19. The procedures used to prevent or reduce contamination during processing should be
19720 validated depending on the type of tissue and how it is processed.

19721 11.3.20. The use of antibiotics during procurement, processing and preservation must be
19722 recorded and the end user should be aware of the use of antibiotics.

19723 11.3.21. Maximum times from procurement until processing and storage must be defined. For
19724 deceased donors, maximum times from circulatory arrest (cardiac arrest or, for organ
19725 donors, cross-clamp time) to procurement must also be defined.

19726 11.3.22. Procurement, processing and storage times must be documented in the records for
19727 tissues and cells.

19728 11.3.23. Tissue establishments must ensure that human tissues and cells are correctly identified
19729 at all times. Each delivery or batch of tissues or cells must be assigned an identifying
19730 code, in accordance with Directive 2004/23/EC Article 8.

19731 11.3.24. Each processed tissue or cell product should have a batch number that is also recorded
19732 in the processing records.

19733 11.3.25. The acceptance, rejection, or disposition of tissues and cells, including those to be
19734 discarded, must be properly documented.

19735 11.4. *Storage*

19736 11.4.1. Maximum storage time and storage conditions must be defined and validated for each
19737 type of tissue and cell.e.

19738 11.4.2. There must be a system in place to separate and distinguish tissues and cells prior to
19739 release/in quarantine from those that are released and from those that are rejected,
19740 in order to prevent mix-ups and cross-contamination..

19741 11.4.3. A documented risk assessment approved by the responsible person must be
19742 undertaken to determine the fate of all stored tissues and cells following the
19743 introduction of any new donor selection or testing criterion or any significantly
19744 modified processing step that enhances safety or quality.

19745 11.5. *Disposal*

19746 11.5.1. Tissue establishments must include in their standard operating procedures special
19747 provisions for the handling of tissues and cells to be discarded, in order to prevent the
19748 contamination of other tissues or cells, the processing environment or personnel.

19749 11.5.2. Procedures for discarding tissue and cells must prevent the contamination of other
19750 donations and tissues and cellss, the processing environment or personnel. These
19751 procedures must comply with national regulations.

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19752 11.5.3. Disposal of human tissues should be carried out in a manner that shows respect for
19753 fundamental rights and for the human body.

19754 11.5.4. For HPC it should be documented that the conditions for disposal defined prior to
19755 procurement have been met, including (where applicable) the option to transfer the
19756 cells to another facility if the designated recipient is still alive after the agreed storage
19757 period.

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19759
12. Packaging, coding and labelling

19760 12.1. *General*

19761 12.1.1. Labelling and packaging operations should be designed to prevent any cross
19762 contamination or mix-ups. Simultaneous operations should be avoided or adequate
19763 measures should be taken to ensure no cross contamination or mix-ups occur.

19764 12.1.2. Facilities where packaging or labelling operations have taken place should be inspected
19765 and documented before starting any other operation so as to guarantee that all the
19766 previous materials have been removed.

19767 12.1.3. There should be written procedures describing: the receipt, identification, quarantine,
19768 sampling, examination and/or testing and release, and handling of packaging and
19769 labelling materials.

19770 12.1.4. Records should be maintained for each shipment of labels and packaging materials
19771 showing receipt, examination, or testing, and whether accepted or rejected.

19772 12.1.5. All packaging and labelling materials should be stored and managed in a safe manner
19773 in order to avoid any cross contamination or mix-up, which could result in incorrectly
19774 identified / packaged tissues / cells.

19775 12.2. *Packaging and labelling*

19776 12.2.1. Containers should provide adequate protection against deterioration or
19777 contamination of the tissues/cells, that may occur during the storage and
19778 transportation conditions, and resist the processing techniques used (e.g.
19779 sterilization).

19780 12.2.2. Containers should be clean and sanitized to ensure that they are suitable for their
19781 intended use. These containers should not alter the quality, safety and efficacy of the
19782 tissues/cells.

19783 12.2.3. Labels should be designed to adhere firmly to the container under all storage and
19784 transport conditions and the processing techniques used.

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19785 12.2.4. Type of label to be used, as well as the labelling methodology, should be defined and
19786 established in written procedures.

19787 12.2.5. Labels applied to containers, equipment or premises should be clear, unambiguous
19788 and in the agreed format of the tissue establishment.

19789 12.2.6. All excess labels should be destroyed or maintained in a secure manner, when
19790 necessary, to prevent cross contamination or mix-ups.

19791 12.2.7. Obsolete labels should be destroyed according to written procedures.

19792 12.2.8. Printed labels should be carefully examined to ensure that information contained
19793 conforms to the corresponding tissue/cells. The results of this examination should be
19794 documented.

19795 12.2.9. A printed label, representative of those used, should be included in the processing
19796 records.

19797 12.2.10. A unique identifying code must be allocated to the donor and the donated tissues and
19798 cells, during procurement or at the end of the recovery process, to ensure proper
19799 identification of the donor and the traceability of all donated material.

19800 12.2.11. Following procurement, all recovered tissues and cells must be packaged in a manner
19801 which minimises the risk of contamination and must be stored at temperatures that
19802 preserve the required characteristics and biological function of the cells/tissues. The
19803 packaging must also prevent contamination of those responsible for packaging and
19804 transportation of the tissues and cells.

19805 12.2.12. The packaged cells/tissues must be shipped in a container which is suitable for the
19806 transport of biological materials and which maintains the safety and quality of the
19807 contained tissue or cells.

19808 12.2.13. Any accompanying tissue or blood samples for testing must be accurately labelled to
19809 ensure identification with the donor, and must include a record of the time and place
19810 the specimen was taken.

19811 12.2.14. *Primary packaging and labelling operation after procurement*

19812 12.2.14.1. At the time of procurement, every package containing tissues and cells must be
19813 labelled. The primary tissue/cell container must indicate the donation identification or
19814 code and the type of tissues and cells.

19815 12.2.14.2. Where the size of the package permits, the following information must also be
19816 provided:

19817 12.2.14.2.1. date (and time where possible) of donation;

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19818 12.2.14.2.2. Blood transfusion before the recovery and haemodilution risk;

19819 12.2.14.2.3. hazard warnings;

19820 12.2.14.2.4. nature of any additives/transport medium (if used);

19821 12.2.14.2.5. in the case of autologous donations, the label must state ‘for autologous use only’;

19822 12.2.14.2.6. in the case of directed donations, the label must identify the intended recipient;

19823 12.2.14.2.7. If any of the information above cannot be included on the primary package label, it
19824 must be provided on a separate sheet accompanying the primary package.

19825 12.2.15. Secondary packaging and labelling operation after procurement

19826 12.2.15.1. When tissues/cells are shipped by an intermediary, every shipping container must be
19827 labelled at least with:

19828 12.2.15.1.1. “TISSUES AND CELLS and HANDLE WITH CARE”;

19829 12.2.15.1.2. the identification of the establishment from which the package is being transported
19830 (address and phone number) and a contact person in the event of problems;

19831 12.2.15.1.3. the identification of the tissue establishment of destination (address and phone
19832 number) and the person to be contacted to take delivery of the container;

19833 12.2.15.1.4. the date and time of the start of transportation;

19834 12.2.15.1.5. specifications concerning conditions of transport relevant to the quality and safety of
19835 the tissues and cells;

19836 12.2.15.1.6. in the case of living tissues and cells, the following indication: DO NOT IRRADIATE;

19837 12.2.15.1.7. when tissues and cells are known to be positive for a relevant infectious disease
19838 marker, the following indication: BIOLOGICAL HAZARD;

19839 12.2.15.1.8. in the case of autologous donors, the following indication: ‘FOR AUTOLOGOUS USE
19840 ONLY’;

19841 12.2.15.1.9. specifications concerning storage conditions (such as DO NOT FREEZE).

19842 12.2.16. *Final labelling for distribution*

19843 12.2.16.1. The primary tissue/cell container must provide:

19844 12.2.16.1.1. type of tissues and cells, identification number or code of the tissue/cells, and lot or
19845 batch number where applicable;

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12.2.16.1.2. identification of the tissue establishment;

12.2.16.1.3. expiry date;

12.2.16.1.4. in the case of autologous donation, this has to be specified (for autologous use only) and the donor/recipient has to be identified;

12.2.16.1.5. in the case of directed donations - the label must identify the intended recipient;

12.2.16.1.6. when tissues and cells are known to be positive for a relevant infectious disease marker, it must be marked as: BIOLOGICAL HAZARD.

12.2.16.2. If any of the information under points 12.2.16.1.4 and 12.2.16.1.5 above cannot be included on the primary container label, it must be provided on a separate sheet accompanying the primary container. This sheet must be packaged with the primary container in a manner that ensures that they remain together.

12.2.16.3. The following information must be provided either on the label or in accompanying documentation:

12.2.16.3.1. description (definition) and, if relevant, dimensions of the tissues;

12.2.16.3.2. morphology and functional data where relevant;

12.2.16.3.3. date of distribution of the tissue/cells;

12.2.16.3.4. biological determinations carried out on the donor and results;

12.2.16.3.5. storage recommendations;

12.2.16.3.6. instructions for opening the container, package, and any required manipulation/reconstitution;

12.2.16.3.7. expiry dates after opening/manipulation;

12.2.16.3.8. instructions for reporting serious adverse reactions and/or events;

12.2.16.3.9. presence of potential harmful residues (e.g. antibiotics, ethylene oxide etc).

12.2.17. External labelling of the shipping container

12.2.17.1. For transport, the primary container must be placed in a shipping container that must be labelled with at least the following information:

12.2.17.1.1. identification of the originating tissue establishment, including an address and phone number;

19874 12.2.17.1.2. identification of the organisation responsible for human application of destination,
19875 including address and phone number;

19876 12.2.17.1.3. a statement that the package contains human tissue/cells and HANDLE WITH CARE;

19877 12.2.17.1.4. where living cells are required for the function of the graft, such as stem cells gametes
19878 and embryos, the following must be added: 'DO NOT IRRADIATE';

19879 12.2.17.1.5. recommended transport conditions (e.g. keep cool, in upright position, etc.);

19880 12.2.17.1.6. safety instructions/method of cooling (when applicable).

19881 12.3. *Coding*

19882 12.3.1. Tissue establishment must have effective and accurate systems to uniquely identify
19883 and label cells/tissues received and distributed. A single European identifying code
19884 must be allocated to all procured tissues and cells, including tissues and cells imported
19885 from countries outside the EU, by the tissue establishment, to ensure proper
19886 identification of the donor and the traceability of all donated material and to provide
19887 information on the main characteristics and properties of tissues and cells.

19888 12.3.2. The code must incorporate at least:

19889 12.3.2.1. Donation identification;

19890 12.3.2.1.1. Unique ID number

19891 12.3.2.1.2. Identification of the tissue establishment

19892 12.3.2.2. Tissues and cells identification;

19893 12.3.2.2.1. Tissues and cells code (basic nomenclature)

19894 12.3.2.2.2. Split number (if applicable)

19895 12.3.2.2.3. Expiry date.

19896

19897 13. Quality control (incl. microbiological control)

19898 13.1. *General*

19899 13.1.1. A quality control system must be in place to ensure that tissues and cells are not
19900 released for use, until their quality has been assessed as satisfactory. Activities, such
19901 as verification steps, sampling and testing should be carried out to assess that the

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19902 tissues and cells, and also materials, equipment, and processes, comply with
19903 established acceptance criteria.

19904 13.1.1.1. All records which are critical to the safety and quality of the tissues and cells must be
19905 protected from unauthorised amendment and kept so as to ensure readability and
19906 access throughout their specified retention period, after expiry date, clinical use or
19907 disposal.

19908 13.1.1.2. Samples for quality control should be representative of the tissues and cells from
19909 which they are taken and should be done and recorded in accordance with written
19910 procedures that describe the method of sampling, including the amount of sample to
19911 be taken, precautions to be observed, storage conditions.

19912 13.1.1.3. At all stages of quality control testing, sampling containers must be labelled with
19913 relevant information on their identity and date of sampling.

19914 13.1.2. There must be a person responsible for quality control, who supervises all quality
19915 control procedures, and ensure that the premises and equipment where quality
19916 control operations are carried out are appropriate and maintained under suitable
19917 conditions and that the personnel working under his/her responsibility is adequately
19918 trained.

19919 13.1.3. Written procedures must be in place that govern quality control at key stages during
19920 processing. The written procedures should include at least:

19921 13.1.3.1. Test method

19922 13.1.3.2. Sample size and sampling plan

19923 13.1.3.3. Accepted criteria

19924 13.1.4. Sampling and testing methods must be validated to show the representativeness of
19925 the sample and the suitability of the selected methods.

19926 13.1.4.1. Performance of the testing procedures should be regularly assessed.

19927 13.1.5. Records related to quality control testing should be part of the tissue and cell
19928 documentation. Data to be recorded and maintained should include:

19929 13.1.5.1. Name, manufacturer and batch number of the material or products used

19930 13.1.5.2. Reference to the relevant specifications and testing procedures, and to equipment
19931 used

19932 13.1.5.3. Test results, including observations and calculations, and reference to any certificates
19933 of analysis

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19934 13.1.5.4. Date of testing

19935 13.1.5.5. Identification of the persons who performed the testing

19936 13.1.5.6. Identification of the person who verified the testing

19937 13.1.5.7. Statement of approval or rejection of the test results

19938 13.1.6. Minimum acceptance criteria should be based on a defined specification for each type
19939 of tissue and cell.

19940 13.1.7. Non-conforming tissues and cells must be identified and separated from conforming
19941 tissues and cells. The fate of non-conforming tissues and cells must be decided by the
19942 responsible person in charge of the tissue establishment, according to written
19943 procedures.

19944 13.2. *Microbiological control*

19945 13.2.1. The microbiological safety of tissues and cells is based on donor selection and
19946 minimisation of initial contamination, with control and monitoring of contamination
19947 during the entire procurement process. All facilities that procure, process or store
19948 tissues and cells should have access to the services of a microbiology laboratory with
19949 a fully implemented quality management system and to the advice of a suitably
19950 qualified expert microbiologist.

19951 13.2.2. Microbiological control should be carried out at least on representative samples of the
19952 tissues and cells before final release, and of tissues and cells at the time of
19953 procurement.

19954 13.2.2.1. In exceptional cases, if sampling of the finished tissues and cells product is not feasible,
19955 storage medium or rinsing or washing solutions can be tested as surrogates.

19956 13.2.2.2. In cases where the nature of the procured tissues and cells does not allow sampling of
19957 the starting material, an alternative sampling approach, such as liquids in contact with
19958 starting material, may be undertaken.

19959 13.2.2.3. When applicable, a sample of the tissues/cells storage, transport or rinsing solution
19960 should be tested.

19961 13.2.2.4. In-process testing should be performed at relevant steps of the preparation process,
19962 according to a risk assessment that has to take into consideration the nature of the
19963 tissues and cells, the origin, procurement, critical steps during processing and their
19964 intended application.

19965 13.2.2.5. For cells such as HPC, in which a closed system is used for processing and where no
19966 further steps are conducted, a reduced testing strategy that relies on single testing of
19967 samples taken at an appropriate time point is applicable.

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19968 19969 19970 19971 13.2.2.6. For processed tissues and cells sterilised in their final container by a validated sterilisation process, if the release is intended to rely on process data only and not on final product testing for sterility, then validated procedures for all critical processing steps and a fully validated sterilisation method must be applied.

19972 19973 19974 19975 13.2.2.7. For tissue and cells obtained from processing that includes decontamination, such as treatment with antibiotics and anti-fungal agents, methods for finishes tissues and cells testing must be evaluated carefully with respect to possible inhibition of microbial growth due to decontaminating agents or their residues.

19976 19977 19978 19979 13.2.3. Microbiological testing methods for the detection of bacteria and fungi should follow the procedures outlined in Chapters 2.6.1, 2.6.27, 2.6.12 or 2.6.13, and General Monography 5.1.6 of the European Pharmacopoeia (Ph. Eur.), according to the method employed and the type of tissue or cell analysed.

19980 19981 13.2.3.1. Independent of the applied method, their suitability must be shown with respect to specificity, sensitivity and robustness.

19982 19983 19984 19985 13.2.3.2. If release of the tissues and cells is necessary before the end of the officially verified/required incubation period, ‘negative-to-date’ reading of the results may be carried out. In this case, intermediate results of the final testing in combination with final results of in-process controls should be used for tissues and cells release.

19986 19987 19988 13.2.3.3. If micro-organisms are detected after tissues and cells release, predefined measures such as identification and antibiotic sensitivity of the species must be carried out and information must be provided to clinicians caring for the patient.

19989 19990 19991 13.2.4. Depending on the type of preparation process, it may be necessary to complement the microbial test concept by additional tests for specific infectious agents such as mycoplasma (Ph. Eur. 2.6.7).

19992 19993 19994 13.2.4.1. Testing should be conducted at manufacturing steps at which mycoplasma contaminations would most likely be detected, such as after pooling or procurement but before washing steps.

19995 19996 13.2.4.2. As mycoplasmas are cell-associated micro-organisms that may locate within the cell, testing should always include the cellular matrix, if possible.

19997 19998 19999 13.2.5. Depending on the intended application of the tissues and cells, and the estimated impact of endotoxins on the recipient, routine testing for endotoxins may be required. If deemed necessary, it should be carried out according to Ph. Eur. 2.6.14.

20000 20001 13.2.5.1. In the case of tissues and cells obtained from culture, an endotoxin test should be carried out on them before release to the patient.

20002 13.2.5.2. In any procedure in which animal derived products are used, endotoxin testing should
20003 be performed.

20004 13.2.5.3. As they are potential sources of endotoxins, raw materials certified to be free of
20005 endotoxins by their manufacturers should be employed in culture of tissues and cells.

20006 13.2.5.4. For certain cells that must be administered immediately and that cannot be
20007 cryopreserved without damaging the viability and quality of cells, a rapid method for
20008 endotoxin testing, that assesses the biological effects as well as the content of
20009 endotoxins, may be employed (Ph. Eur. 2.6.30).

20010 13.2.6. Each batch of the microbiological culture medium and plates to be used must be tested
20011 for its growth-promoting capacities – a 'growth-promotion test' in accordance with Ph.
20012 Eur. 2.6.1 and 2.6.27.

20013 13.2.7. The methods employed for microbiological testing must be validated in the presence
20014 of the intended sample material (method suitability test). The method suitability test
20015 must be carried out using the bacterial and fungal species indicated in Ph. Eur. 2.6.1.

20016 13.2.8. Source material that demonstrates contamination must be rejected unless the
20017 preparation undergoes decontamination and/or terminal sterilisation, and the
20018 detected quantity and quality of micro-organisms can be reliably inactivated or
20019 removed by the intended procedure, or if it is justified by exceptional clinical
20020 circumstances.

20021

20022 14. Distribution, Import / Export and Recall

20023 14.1. *Release*

20024 14.1.1. The distribution process, meant as transportation and delivery of cells or tissues
20025 intended for human application, or for use in further manufacture, must be validated.

20026 14.1.2. Prior to distribution a comprehensive record review must ensure that all elements of
20027 procurement, processing and storage have met the established quality criteria
20028 including identity of the tissues and cells.

20029 14.1.3. Packaged tissues or cells should be examined visually for appropriate labelling and
20030 container integrity.

20031 14.1.4. Tissues and cells should not be distributed without an order from a physician or other
20032 authorised health professional.

20033 14.1.5. In case of incomplete eligibility of the donor the tissues and cells must be released only
20034 for documented urgent medical need and after a risk assessment has been performed.

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20035 14.1.6. Distribution for clinical application must be restricted to authorized organisation
20036 responsible for human application or to authorized health professional and must
20037 comply with all applicable national laws and regulations. In case tissues or cells require
20038 additional procedures such as thawing to be undertaken by the ORHA, the associated
20039 specific instructions must be provided to the ORHA by the tissue establishment.

20040 14.2. *Transport*

20041 14.2.1. Equipment used to ensure the maintenance of critical transport or shipment must be
20042 qualified.

20043 14.2.2. The capacity of the transport container to maintain the required environmental
20044 conditions and the length of time that these conditions can be maintained should be
20045 determined by validation and documented.

20046 14.2.3. The container / package must be secure, shipment conditions such as temperature and
20047 time limit must be defined to ensure maintenance of the required properties of tissues
20048 and cells.

20049 14.2.4. Data loggers or temperature indicators must be used when it is mandatory to monitor
20050 temperature during transport or shipment of tissues or cells requiring a continuous
20051 temperature controlled environment.

20052 14.2.5. The mode of transport or shipment must comply with the applicable laws and
20053 regulations on transportation of biological substances.

20054 14.2.6. An alternative plan of transport or shipping should be available in case of emergency
20055 situations to prevent possible clinical complications to the recipient.

20056 14.2.7. A courier should be able to contact the receiving facility on a 24-hour basis in case of
20057 delay during transit.

20058 14.2.8. Viable tissues and cells, including stem cells, gametes and embryos, must not be
20059 exposed to irradiation. Appropriate arrangements in accordance with relevant
20060 national legislation should be in place to ensure that such tissues and cells are not
20061 exposed to irradiation during transport, including at security screenings and border
20062 crossings.

20063 14.3. *Agreements*

20064 14.3.1. Written agreements must be in place for the shipment of tissues and cells between
20065 the shipping company and the tissue establishment.

20066 14.3.2. A service level agreement between the exporting and importing TE must clearly define
20067 roles and responsibilities including procedures of transport, packaging and required
20068 environmental conditions.

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20069 14.3.3. The agreement should specify how tissues and cells will be identified, a unique
20070 identifying code must allow unambiguous identification and traceability.

20071 14.4. *Export*

20072 14.4.1. Exported tissues and cells must be procured, handled, stored, transported, used and
20073 disposed of in accordance with the consent that have been given by the donor.

20074 14.4.2. Tissues and cells should be exported only to countries that have proper controls on the
20075 use of donated material and only for the purposes for which they can lawfully be used
20076 in the country of destination.

20077 14.5. *Import*

20078 14.5.1. Tissues establishments must be authorized for the import of tissues and cells from
20079 non-EU countries by their respective Health Authorities.

20080 14.5.2. The importing TE should assess and document that the exporting TE apply the
20081 fundamental ethical principles of consent, non-remunerated donation, anonymity,
20082 respect for public health.

20083 14.5.3. The importing TE must evaluate the general quality and safety systems at the exporting
20084 establishment, licences and accreditations and the donor blood testing.

20085 14.5.4. The importing TE must require that any changes to authorisation status be
20086 immediately communicated by the exporting facility.

20087 14.5.5. The agreements between an importing TE and suppliers in other non-EU countries
20088 should include provisions for the performance of audits at the exporting facility.

20089 14.5.6. Acceptance at the TE should include a documented procedure to verify compliance
20090 with the written agreement in place with the exporter.

20091 14.5.7. Containers should be examined for any evidence of tampering or damage during
20092 transport.

20093 14.5.8. Tissues and cells should be stored in quarantine in an appropriate secure location
20094 under defined conditions until they, along with the accompanying documentation
20095 have been verified as conforming to requirements.

20096 14.5.9. The importing TE must identify and code the imported tissues and cells with the
20097 appropriate Single European Code (SEC).

20098 14.6. *Records*

20099 14.6.1. The courier must provide records of pick-up and delivery to the TE to ensure a
20100 complete traceability of the tissues and cells.

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20101 14.6.2. Documentation obtained from the exporting tissue establishment must be archived
20102 for the time period required by national regulations (e.g., 30 years in EU member
20103 States).

20104 14.7. *Recall*

20105 14.7.1. The TE must have personnel authorized to assess the need for recall and to initiate and
20106 coordinate the necessary actions.

20107 14.7.2. A recall procedure must be in place including a description of the responsibilities,
20108 actions to be taken, within pre-defined periods of time, and notification to the Health
20109 Authorities.

20110 14.7.3. Actions must be taken within pre-defined periods of time and must include tracing all
20111 relevant tissues or cells and, where applicable, must include trace-back. The purpose
20112 of the investigation is to identify any donor who might have contributed to causing the
20113 adverse reaction and to retrieve available tissues or cells from that donor, as well as
20114 to notify consignees and recipients of components procured from the same donor in
20115 the event that they might have been put at risk.

20116 14.7.4. The progress of the recall process should be recorded and a final report issued,
20117 including reconciliation of the delivered and recovered quantities of the tissues or
20118 cells.

20119 14.7.5. The effectiveness of the arrangements for recalls should be regularly evaluated.

20120

20121 # 15. Documentation

20122 15.1. *General principles*

20123 15.1.1. Good documentation constitutes an essential part of the Quality System and is key to
20124 operating in compliance with Good Practice requirements. Various types of documents
20125 and media used must be defined fully in the QMS of the organisation.

20126 15.1.2. Documentation may exist in various forms: paper-based, electronic or photographic.
20127 The main objective of the system of documentation used must be to establish, control,
20128 monitor and record all activities that directly or indirectly impact on all aspects of the
20129 quality and safety of tissues and cells as well as any derived medicinal products. The
20130 Quality Management System must include sufficient instructional detail to facilitate
20131 common understanding of the requirements, in addition to providing for adequate
20132 recording of the various processes and evaluation of any observations, so that ongoing
20133 application of the requirements may be demonstrated.

20134 15.1.3. There are two primary types of documentation used to manage and record Good

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Practice compliance: instructions (directions, requirements) and records/reports. Appropriate practices should be applied with respect to the type of document. Suitable controls must be implemented to ensure the accuracy, integrity, availability and legibility of documents. Instruction documents must be free from errors and available in writing. The term ‘written’ means recorded or documented on media from which data may be rendered in a readable form for humans.

15.2. *Required good practice documentation*

15.2.1. Documentation must include at least the following items:

15.2.1.1. a quality manual;

15.2.1.2. specifications for materials and reagents;

15.2.1.3. approved SOP for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;

15.2.1.4. identification and analysis of risks and a risk mitigation plan;

15.2.1.5. records on the performance of operations, including processing records;

15.2.1.6. records of deviations, complaints and audits;

15.2.1.7. training and competency records of personnel;

15.2.1.8. qualitative and quantitative specifications for tissues and cells;

15.2.1.9. key quality indicators for tissues and cells.

15.2.2. *Instructions (directions or requirements*).

15.2.2.1. Specifications based on policies and risk assessments describe in detail the requirements to which the tissues and cells or other materials used or obtained during processing and distribution must conform.

15.2.2.2. Testing instructions detail all the starting materials, equipment and computerised systems (if any) to be used and specify all sampling and testing instructions. If applied, in-process controls must be specified, together with their acceptance criteria.

15.2.2.3. Procedures (otherwise known as Standard Operating Procedures or SOPs) give directions for performing certain operations.

15.2.2.4. Standard operating procedures (SOPs) provide explicit instructions for performing certain discreet operations, and may record the outcome (e.g. qualification and validation protocols).

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15.2.2.5. Technical agreements are agreed between contract givers and acceptors for outsourced activities.

15.2.3. *Records/reports*

15.2.3.1. Records provide evidence of various actions taken to demonstrate compliance with instructions, e.g. activities, events, investigations and a history of all tissues and cells, including their distribution. Records include the raw data that is used to generate other records. For electronic records, regulated users should define which data are to be used as raw data. All data on which quality decisions are based should be defined as 'raw data'.

15.2.3.2. Certificates of analysis provide a summary of testing results on samples of reagents, products or materials, together with the evaluation for compliance with a stated specification.

15.2.3.3. Reports document the carrying out of particular exercises, projects or investigations, together with results, conclusions and recommendations.

15.3. *Generation and control of documentation*

15.3.1. All types of documents should be defined and adhered to. Requirements apply equally to all forms of document media types. Complex systems must be understood, well documented and validated, and adequate controls must be in place. Many documents (instructions and/or records) may exist in hybrid forms (i.e. some elements are electronic and others are paper-based). Relationships and control measures for master documents, official copies, data handling and records must be stated for both hybrid and homogenous systems.

15.3.2. A document control system, defined in a written procedure, must be established for the review, revision history and archiving of documents, including SOPs. Appropriate controls for electronic documents, such as templates, forms and master documents, must be implemented. Appropriate controls must be in place to ensure the integrity of the record throughout the retention period.

15.3.3. Documents should be designed, prepared, reviewed, and distributed with care. Reproduction of working documents from master documents should not allow errors to be introduced through the reproduction process.

15.3.4. There must be a document control procedure in place to ensure that only current versions are in use.

15.3.5. Documents containing instructions must be approved, signed and dated by appropriate and authorised persons. This may also be undertaken electronically. Documents should have unambiguous content and be uniquely identifiable. The effective date must be defined.

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20206 15.3.6. Documents containing instructions should be laid out in an orderly fashion and be easy
20207 to check. The style and language of documents should fit with their intended use. SOP,
20208 Work Instructions and Methods should be written in an imperative mandatory style.

20209 15.3.7. Documents within the QMS must be regularly reviewed and kept up-to-date. A
20210 periodic review process should be established to ensure that the documentation for
20211 any given process, system or equipment is complete, current and accurate.

20212 15.3.8. All changes to documents must be reviewed, dated, approved, documented and
20213 implemented promptly by authorised personnel.

20214 15.3.9. Instructional documents should not be hand-written; although, where documents
20215 require the entry of data, sufficient space should be provided for such entries.

20216 15.4. *Good documentation practices*

20217 15.4.1. Records must be legible and may be handwritten, transferred to another medium such
20218 as microfilm, or documented in a computerised system.

20219 15.4.2. Records should be made or completed at the time each action is taken and in such a
20220 way that all significant activities concerning the coding, donor eligibility, procurement,
20221 processing, preservation, storage, transport, distribution or disposal, including aspects
20222 relating to quality control and quality assurance of tissues and cells are traceable.

20223 15.4.3. For every critical activity, the materials, equipment and personnel involved must be
20224 identified and documented.

20225 15.4.4. The record system must ensure continuous documentation of the procedures
20226 performed from the donor to the recipient. That is, each significant step must be
20227 recorded in a manner that permits tissue and cells or procedure to be traced, in either
20228 direction, from the first step to final use/disposal.

20229 15.4.5. Any alteration made to the entry on a document must be signed and dated; the
20230 alteration must permit reading of the original information. Where appropriate, the
20231 reason for the alteration should be recorded. In case of electronic records, there must
20232 be an audit trail, so that it is traceable as to what data has been altered, when it was
20233 altered and who altered it.

20234 15.4.6. Access to records (registers and data) must be restricted to persons authorised by the
20235 responsible person, and to the health authority for the purpose of inspection and
20236 control measures.

20237 15.4.7. Data protection and confidentiality measures must be in place, in accordance with
20238 Article 14 of Directive 2004/23/EC.

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20239 15.5. *Retention of documents*

20240 15.5.1. It should be clearly defined which record is related to each activity and where this
20241 record is located. Secure controls must be in place to ensure the integrity of the record
20242 throughout the retention period. These controls must be validated if appropriate.

20243 15.5.2. Records encompassing identification, donor tests and clinical evaluation of the donor
20244 must be retained an include at least the following details:

20245 15.5.2.1. Identification;

20246 15.5.2.2. Age;

20247 15.5.2.3. Sex;

20248 15.5.2.4. medical and behavioural history;

20249 15.5.2.5. relevant clinical data, laboratory test results, and results of any other tests;

20250 15.5.2.6. outcome of physical examination, results of autopsy (if carried out) or preliminary
20251 verbal report for deceased donors;

20252 15.5.2.7. completed haemodilution algorithm (where applicable);

20253 15.5.2.8. consent/authorization forms;

20254 15.5.2.9. for HPC donors, report of donor's suitability for intended recipient and, if donor is
20255 unrelated, relevant donor data to confirm suitability.

20256 15.5.3. Donor testing records that must be accessible at the laboratory and include date and
20257 time of sampling, date and time of sample receipt at the testing facility, record of test
20258 kits used to test donor sample, results of donor testing including repeat testing

20259 15.5.4. Records of procurement of tissues and cells must be retained. A procurement report
20260 should be available that includes:

20261 15.5.4.1. identification of procurement organization, and person responsible for procurement,
20262 including signature;

20263 15.5.4.2. identification of tissue establishment receiving the tissue/cells;

20264 15.5.4.3. donor identification data (including how and by whom the donor was identified);

20265 15.5.4.4. donation unique number;

20266 15.5.4.5. date, time and place of donation, and standard operating procedure used for
20267 procurement;

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20268 15.5.4.6. type of donation;

20269 15.5.4.7. description of the procurement area, including environmental conditions;

20270 15.5.4.8. storage conditions for deceased donors (including whether refrigeration was applied,
20271 and time of start and end of refrigeration);

20272 15.5.4.9. details on materials, reagents and transport solutions;

20273 15.5.4.10. incidents during procurement.

20274 15.5.5. Records of processing, storage and distribution of tissues and cells must be retained.
20275 A processing report should be available that at least includes the details of:

20276 15.5.5.1. tissues and cells received and evaluation of their suitability;

20277 15.5.5.2. SOP used to process the tissues and cells;

20278 15.5.5.3. equipment used during processing;

20279 15.5.5.4. materials used during processing;

20280 15.5.5.5. sterilisation or decontamination;

20281 15.5.5.6. cryopreservation and freezing protocols;

20282 15.5.5.7. environmental monitoring;

20283 15.5.5.8. tissues and cells testing, including microbiological testing;

20284 15.5.5.9. incidents that occurred during processing;

20285 15.5.5.10. Storage and distribution; reports should be available that detail:

20286 15.5.5.11. storage location (and transfer record if location is changed);

20287 15.5.5.12. date placed in storage and removed from storage;

20288 15.5.5.13. storage temperature;

20289 15.5.5.14. incidents that occurred during storage;

20290 15.5.5.15. name of party responsible for distribution;

20291 15.5.5.16. identification of establishment, courier or individual who transports tissues/cells at
20292 any stage between procurement and end use;

20293 15.5.5.17. packaging;

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20294 15.5.5.18. time and date of distribution and delivery;

20295 15.5.5.19. identification of receiving establishment, clinician or ORHA;

20296 15.5.5.20. incidents that occurred during distribution

20297 15.5.6. Records of clinical application of tissue and cells should be retained by ORHA and
20298 should include:

20299 15.5.6.1. identification of supplier TE;

20300 15.5.6.2. identification of clinician or ORHA;

20301 15.5.6.3. type of tissues and cells;

20302 15.5.6.4. tissues and cells identification;

20303 15.5.6.5. identification of the recipient;

20304 15.5.6.6. date of clinical application;

20305 15.5.6.7. incidents that occurred during clinical applications;

20306 15.5.6.8. adverse reactions in the recipient;

20307 15.5.6.9. health outcomes of children born following MAR

20308 15.5.7. Specific retention requirements for certain documentation apply.

20309 15.5.7.1. Records must be retained for a period according to local, national or EU requirements,
20310 as appropriate.

20311 15.5.7.2. Traceability data (that allow tracing from donor to recipient and *vice versa*) must be
20312 retained for a minimum of 30 years.

20313 15.5.7.3. All records, including raw data, which are critical to the safety and quality of the tissues
20314 and cells must be kept for at least 10 years after expiry date, clinical use or disposal.

20315 15.5.7.4. Quality System documentation and associated records should be retained for a
20316 minimum of 10 years.

20317 15.5.7.5. For other types of documentation, the retention period should be defined on the basis
20318 of the business activity that the documentation supports. These retention periods
20319 should be specified.

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20322 # 16. Traceability

20323 ## 16.1. *General*

20324–20331 16.1.1. A robust system must be established and maintained to trace a specific tissue/cell during any step from donor/donation to recipient or child conceived as a result of MAR treatment, in a bidirectional way. In the EU, tissues and cells are traceable from procurement to human application or disposal and vice versa through documentation and the use of the Single European Code (SEC) (Directive 2006/86/EC as amended by Commission Directive EU 2015/565). Tissues and cells used for advanced therapy medicinal products must be traceable under this Directive at least until transferred to the Advanced Therapy Medicinal Product manufacturer.

20332–20333 16.1.2. All relevant data relating to products and materials coming into contact with tissues and cells must also be traceable.

20334–20338 16.1.3. Each organization holding tissues or cells must have effective and accurate procedures to uniquely identify and label cells/tissues collected, received, processed, distributed/disposed and used for human application. The application of SEC does not preclude the additional application of other codes in accordance with Member States' national requirements.

20339–20341 16.1.4. For accurate transcription of critical identification information, electronic transfer should be used. If manual transcription is used, double checking of data should be implemented.

20342–20345 16.1.5. Responsibility for traceability among the different organizations involved in procurement, processing and distribution, and human application of cells/tissues must be clearly defined. Responsibility should be defined in a written technical and legal agreement.

20346–20350 16.1.6. Traceability data must be kept long-term after clinical use to allow adequate biovigilance and follow-up. In the EU, information related to traceability, as described in Annex VI of the Directive 2006/86/EC, must be retained for at least 30 years after application or cell/tissue expiry date. Data that are critical to the safety and quality of cells/tissues should be maintained for at least 10 years.

20351–20353 16.1.7. Traceability data must be stored securely in an appropriate archive. In the case of change of storage location, a link between the previous location and new location must be established.

20354–20355 16.1.8. Audits of traceability from donor to recipient and vice versa must be included in the quality management plan.

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17. Biovigilance

17.1. *General*

17.1.1. Tissues establishments must have documented procedures in place for the reporting of serious adverse events and serious adverse reactions (SARE) as defined in Directive 2004/23/EC

17.1.2. There should be systems in place to ensure that adverse events, adverse reactions and near misses are documented, carefully investigated and where necessary, followed up by the implementation of corrective actions to prevent recurrence.

17.1.3. Systems must be in place to assure the follow-up of tissue recipients and children conceived after MAR treatments

17.1.4. There should be procedures in place for reporting SARE in a timely manner to the Responsible Person for the TE and Health Authorities. Adequate resource must be made available for their immediate investigation, resolution and implementation of any corrective and preventive actions.

17.1.5. There should be a coordinator, who has responsibility for vigilance and surveillance specified in their job description.

17.1.6. Vigilance programmes should include an activity of scanning for new risks that have not been recognised previously. New risks may be related to donors, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing.

17.1.7. Newly emerging infectious diseases, for which targeted testing can be carried out or which might imply the need to exclude certain donors, represent an example of one type of new risk.

17.1.8. Co-ordination between various systems of vigilance (e.g. organ transplantation, medical devices vigilance, pharmacovigilance) should be in place at the local level (tissue establishment) and at the Health Authority level.

17.1.9. Effective communication of the results of vigilance systems is fundamental to ensuring that the benefits of these programmes are realised in practice. Regular feedback to healthcare professionals is critical to support continued notification of adverse reactions and events.

17.1.10. Tissue establishments and clinicians should promote a culture that encourages reporting in a non-punitive context for the benefit of patients and donors. It should be accepted that mistakes do happen and that the human application of tissues and cells is not risk free.

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20391 17.1.11. Programmes of training and awareness should be organised to encourage reporting.
20392 The message that reporting and dissemination vigilance and surveillance information
20393 can result in positive improvements for donors and patients should be promoted.

20394 17.2. *Adverse reactions*

20395 17.2.1. Adverse reactions must be detected, reported, investigated and assessed in terms of
20396 severity, imputability, probability of recurrence or frequency, and consequences.

20397 17.2.2. Efficient systems for rapid quarantine and recall of unsafe tissues or cells must be in
20398 place, along with procedures for look-back where donors or recipients are found to
20399 have been exposed to a risk.

20400 17.2.3. Important outcomes from each adverse reaction should be disseminated
20401 appropriately.

20402 17.2.4. The following are examples of reportable adverse reactions [with abbreviated
20403 descriptions in square brackets]:

20404 17.2.4.1. suspected harm in living donor related to procurement [donor harm];

20405 17.2.4.2. unexpected primary infections possibly transferred from donor to recipient [e.g. viral,
20406 bacterial, parasitic, fungal, prion) [infection from donor]

20407 17.2.4.3. suspected transmitted infection (viral bacterial, parasitic, fungal, prion) possibly due
20408 to contamination or cross-contamination by an infectious agent in the procured
20409 tissues, cells or associated materials, between procurement and their clinical
20410 application [infection from infected/contaminated tissues and cells];

20411 17.2.4.4. unexpected hypersensitive reactions, including allergy, anaphylactoid reactions or
20412 anaphylaxis [hypersensitivity];

20413 17.2.4.5. malignant disease possibly transferred by the tissues or cells (donor-derived, process-
20414 associated or other) [malignancy];

20415 17.2.4.6. unexpected delayed or absent engraftment or graft failure (including mechanical
20416 failure) [failure];

20417 17.2.4.7. toxic effects to tissues and cells or associated materials [toxicity];

20418 17.2.4.8. unexplained immunological reactions due to tissue or cell mismatch [mismatch];

20419 17.2.4.9. aborted procedure involving unnecessary exposure to risk e.g. wrong tissue supplied,
20420 discovered after patient is anaesthetised and the surgical procedure has begun [undue
20421 risk];

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20422 17.2.4.10. suspected transmission of genetic disease by transplantation or gamete/embryo
20423 donation [genetic abnormality];

20424 17.2.4.11. suspected transmission of other (non-infectious) illness [other transmission];

20425 17.2.4.12. transfusion-associated circulatory overload in haematopoietic progenitor
20426 transplantation [volume overload];

20427 17.2.4.13. neurological reaction [insult];

20428 17.2.4.14. severe febrile reaction [fever];

20429 17.2.4.15. other [other].

20430 17.2.5. The tissue establishment is responsible for providing clinical-user entities,
20431 procurement organisations and critical third parties with clear instructions, forms and
20432 guidance on how to notify adverse reactions in accordance with national and local
20433 requirements.

20434 17.2.6. If serious adverse reactions are detected in relation to tissues or cells that have
20435 entered international distribution channels, appropriate international collaboration
20436 should ensure that all those involved (clinicians, tissue establishments and Health
20437 Authorities) in each of the countries concerned are informed and participate, if
20438 necessary, in the investigation and follow up.

20439 17.3. *Adverse events*

20440 17.3.1. Adverse events can occur at any moment from donor selection to clinical application.
20441 For effective detection of adverse events, all relevant parties must be aware of their
20442 responsibilities for identifying errors or unexpected results. This includes all staff at
20443 tissue establishments and procurement organisations, those working in organisations
20444 such as testing laboratories that provide "third party" services to tissue
20445 establishments, and clinical users who may also detect errors at the point of clinical
20446 use. In EU Directive 2006/86/EC, the definition of a serious adverse event includes
20447 those incidents often referred to as "near misses", i.e. where an error or fault is
20448 detected and corrected without causing harm.

20449 17.3.2. Deviations from requirements of the quality system should be documented and
20450 investigated as part of the internal quality management system. On occasions,
20451 however, a deviation may be of such importance that it should be considered a serious
20452 adverse event and reported through the vigilance system.

20453 17.3.3. According to instructions from the European Commission and EU member states for
20454 annual vigilance reporting, deviations from SOPs in tissue establishments (or other
20455 adverse events) that have implications for the quality and safety of tissues and cells

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20456 should result in serious adverse event reporting to the Health Authority if one or more
20457 of the following criteria apply:

20458 17.3.3.1. inappropriate tissues or cells have been disturbed for clinical use, even if not used;

20459 17.3.3.2. aborted procedure involving unnecessary exposure to risk e.g. wrong tissue supplied,
20460 discovered after patient is anaesthetised and the surgical procedure has begun [undue
20461 risk];

20462 17.3.3.3. the event could have implications for other patients or donors because of shared
20463 practices, services, supplies or donors;

20464 17.3.3.4. the event has resulted in a mix-up of gametes or embryos;

20465 17.3.3.5. the event has resulted in a loss of traceability of tissues or cells;

20466 17.3.3.6. the event resulted in a loss of any irreplaceable autologous tissues or cells or any highly
20467 matched (i.e. recipient specific) allogeneic tissues or cells;

20468 17.3.3.7. the event resulted in loss of a significant quantity of allogeneic tissues or cells.

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20475 # LIST OF THE APPENDICES

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20477 # Appendix 1. General reference documents used

20478 The experts who developed the chapters in this Guide incorporated principles and specific text from
20479 many regulatory, professional and scientific publications. The following are the principal reference
20480 documents used.

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20485 tissues for transplantation, World Health Organization, available at
20486 www.who.int/entity/transplantation/AM-SafetyEssential%20HCTT.pdf, accessed 20 January
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20488 • American Association of Tissue Banks. Standards for tissue banking of the American Association
20489 of Tissue Banks. 14th edition. McLean VA, USA: American Association of Tissue Banks.

20490 • Commission Decision 2010/453/EU of 3 August 2010 establishing guidelines concerning the
20491 conditions of inspections and control measures, and on the training and qualification of officials,
20492 in the field of human tissues and cells provided for in Directive 2004/23/EC of the European
20493 Parliament and of the Council, available at http://eur-lex.europa.eu/legal-
20494 content/EN/TXT/PDF/?uri=CELEX:32010D0453&from=EN, accessed 20 January 2019.

20495 • Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the
20496 European Parliament and of the Council as regards certain technical requirements for the donation,
20497 procurement and testing of human tissues and cells, available at http://eur-
20498 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:038:0040:0052:EN:PDF, accessed 20
20499 January 2019.

20500 • Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the
20501 European Parliament and of the Council as regards traceability requirements, notification of
20502 serious adverse reactions and events and certain technical requirements for the coding, processing,
20503 preservation, storage and distribution of human tissues and cells, available at http://eur-
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- Council of Europe (2005) Convention on Action against Trafficking in Human Beings, and its Explanatory Report, available at http://conventions.coe.int/treaty/en/Treaties/Html/197.htm, accessed 20 January 2019.

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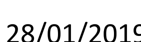

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Appendix 2. Acronyms and other abbreviations

3D	three-dimensional
AATB	American Association of Tissue Banks
AAV	*adeno*-associated virus
Ab	antibodies
ACI	autologous chondrocyte implantation
AFC	antral follicle count
AIDS	acquired immunodeficiency syndrome
ALK	anterior lamellar keratoplasty
AM	amniotic membrane
AMH	Anti-Müllerian hormone
Anti-CMV	antibody to *Cytomegalovirus*
Anti-EBV	antibody to Epstein–Barr virus
Anti-HBc	antibody to hepatitis B core antigen
Anti-HCV	antibody to hepatitis C virus
Anti-HIV-1	antibody to HIV-1
Anti-HIV-2	antibody to HIV-2
ARE	adverse reaction or event
ART	assisted reproductive technology
ARTHIQS	Assisted Reproductive Technologies and Haematopoietic stem cells for transplantation Improvements for Quality and Safety throughout Europe [joint action]
ATMP	advanced therapy medicinal product
ATP	adenosine triphosphate
aW	available water
BET	bacterial endotoxin test
BFU-E	burst-forming units erythroblast
BM	bone marrow
BMDW	Bone Marrow Donors Worldwide [organisation]
BMP	bone morphogenetic proteins
BMSC	bone marrow stromal cells
BSS	balanced salt solution
CAPA	corrective and preventive action
CAR	chimeric antigen receptor
CAT	Committee for Advanced Therapies
CBC	complete blood count
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* infection
CD-P-TO	European Committee (Partial Agreement) on Organ Transplantation of the Council of Europe
CE (marked)	Conformité Européenne
CEA	cultured epithelial autografts
CFU	colony-forming units
CFU-GM	colony-forming units-granulocyte/monocyte
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate
CJD	Creutzfeldt–Jakob disease
CLET	cultivated limbal epithelial transplantation

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CMV	*Cytomegalovirus*
CNS	central nervous system
CNT	Centro Nazionale Trapianti (Italy)
COC	cumulus enclosed oocyte
COD	cause of death
COHS	controlled ovarian hyperstimulation
COMET	cultivated oral mucosal epithelial transplantation
DALK	deep anterior lamellar keratoplasty
DBD	deceased by brain death
DBM	demineralised bone matrix
DBO	Department of Biological Standardisation, OMCL Network & HealthCare (at the European Directorate for the Quality of Medicines & HealthCare)
DC	dendritic cells
DCD	deceased by circulatory death
DH-BIO	Committee on Bioethics of the Council of Europe
DLI	donor lymphocyte infusions
DM	Diabetes mellitus
DMEK	Descemet membrane endothelial keratoplasty
DMSO	dimethyl sulphoxide
DQ	design qualification
DSAEK	Descemet stripping automated endothelial keratoplasty
DVT	deep-vein thrombosis
EATB	European Association of Tissue Banks
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein–Barr virus
EC	European Commission
ECCTR	European Cornea and Cell Transplant Registry
ECDC	European Centre for Disease Prevention and Control
ECM	extracellular matrix
ECVAM	European Centre for the Validation of Alternative Methods
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	ethylenediamine tetra-acetic acid
EEBA	European Eye Bank Association
EGF	endothelial growth factor
EGTA	ethylene glycol tetra-acetic acid
EIA	enzyme immunoassay
EK	endothelial keratoplasty
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMDIS	European Marrow Donor Information System
EQSTB	European Union project 'European Quality System for Tissue Banking'
ESBL	extended-spectrum beta lactamases
ESC	embryonic stem cell
ESHRE	European Society for Human Reproduction and Embryology
EU	European Union *also* endotoxin units
EUROCET	European Registry for Organs, Tissues and Cells
Eurocode IBLS	Eurocode International Blood Labelling Systems

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EuroGTP	Euro Good Tissue Practices [EU project]
EuroGTP-II	Good [Tissue] Practices for demonstrating safety and quality through recipient follow-up [EU project]
EUSTITE	European Standards and Training in the Inspection of Tissue Establishments [EU project]
EUTC	European Code for Tissues and Cells
FACS	fluorescence-activated cell sorting
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration (USA)
FED	Fuchs endothelial dystrophy
FIPS	fingerprints
FMEA	failure mode and effects analysis
FMECA	failure mode, effects and criticality analysis
FMT	faecal microbiota transplantation
FNHTR	febrile non-haemolytic reactions
FOS	fastidious organism supplement
FSH	follicle-stimulating hormone
GAG	glycosaminoglycans
G-CSF	granulocyte-colony stimulating factor
GEMM	granulocyte-erythrocyte-macrophage-megakaryocyte
GF	growth factors
GM	granulocytes and macrophages
GM-CSF	granulocyte macrophage-colony stimulating factor
GMP	Good manufacturing practice [EU document]
GPA	glycerol-preserved allografts
GPG	Good practice guidelines for tissue establishments: Part E of this Guide
GTP	good tissue practice
GV	germinal vesicle
GvHD	graft-*versus*-host disease
GvT	graft-*versus*-tumour
HACCP	hazard analysis and critical control points
hAM	human amniotic membrane
HAV	hepatitis A virus
HBc	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HEPA	high-efficiency particulate air
HES	hydroxyethyl starch
HHV	human *Herpes* virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMG	human menopausal gonadotrophin
HPC	haematopoietic progenitor cells
HPV	human *papilloma* virus
HRV	human *rotavirus*
HSC	haematopoietic stem cells
HSG	hysterosalpingography
HSV	*Herpes simplex* virus
HTLV	human T-lymphotrophic virus
HVAC	heating, ventilating, and air conditioning
HyCoSy	hysterosalpingo-contrast sonography
IATA	International Air Transport Association

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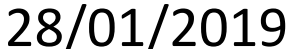

ICCBBA	International Council for Commonality in Blood Banking Automation
ICMART	International Committee Monitoring Assisted Reproductive Technologies
ICSI	intracytoplasmic sperm injection
ICU	intensive care unit
IDM	infectious disease marker
IEC	Independent Ethics Committee
IFN	interferon
Ig	immunoglobulin
IL	interleukin
iPSC	induced pluripotent stem cell
IQ	installation qualification
ISCT	International Society for Cellular Therapy
ISN	International Society for Nephrology
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineering
ISSCR	International Society for Stem Cell Research
IT	information technology
IUI	intra-uterine insemination
IVF	*in vitro* fertilisation
IVM	*in vitro* maturation
JACIE	Joint Accreditation Committee–ISCT & EBMT
KIR	killer immunoglobulin-like receptors
KLAL	keratolimbal allograft
KPI	key performance indicator
LAL	limulus amoebocyte lysate
LH	luteinising hormone
LPS	lipopolysaccharide
LSC	limbal stem cells
MAR	medically assisted reproduction
MCM	metastatic cutaneous melanoma
MESA	microsurgical epididymal sperm aspiration
MII	metaphase II
MNC	mononuclear cells
MPHO	medical products of human origin
MRA	marrow re-populating ability
MRSA	methicillin-resistant *Staphylococcus aureus*
MSC	mesenchymal stromal (stem) cells
MTT	tetrazolium salt assay
NAC	nipple–areola complex
NAT	nucleic acid amplification technique/nucleic acid test
NEC	necrotising enterocolitis
NICE	National Institute of Health and Clinical Excellence
NK	natural killer
NRT	neutral red test
NtPSC	nuclear-transfer pluripotent stem cells
OA	osteoarthritis
OECD	Organisation for Economic Co-operation and Development
OHSS	ovarian hyperstimulation syndrome
ONT	Organización Nacional de Trasplantes (Spain)
OQ	operational qualification
ORHA	organisation responsible for human application

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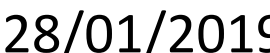

Parvo-B19	*parvovirus* B19
PBK	pseudophakic bullous keratopathy
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
PESA	percutaneous epididymal sperm aspiration
PGD	pre-implantation genetic diagnosis, *see* PGT
PGS	pre-implantation genetic screening, *see* PGT
PGT	pre-implantation genetic testing (formerly known as PGD and PGS)
PGT-A	pre-implantation genetic screening for aneuploidy screening
PGT-M	pre-implantation genetic diagnosis for monogenic/single gene defects
PGT-SR	pre-implantation genetic diagnosis for chromosomal structural re-arrangements
Ph. Eur.	*European Pharmacopoeia*, 9th edn, Strasbourg: Council of Europe 2016
PIC/S	Pharmaceutical Inspectorate Co-operation Scheme
PK	penetrating keratoplasty
PN	ProNucleus
PO	procurement organisation
POI	premature ovarian insufficiency
POSEIDON	Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe [EU project]
PQ	performance qualification
PRF	platelet-rich fibrin
PRIVILEGED	Privacy in Law, Ethics and Genetic Data [EU project]
PROH	1,3-propanedial
PRP	platelet-rich plasma
PVP	PolyVinylPyrrolidone
QC	quality control
QM	quality manager
QMS	quality management system
QRM	quality risk management
RABS	restricted access barrier system
RANTES	regulated on activation, normal T-cell expressed and secreted
RATC	rapid alerts for tissues and cells
RBC	red blood cell
RCT	randomised control trial
RhD	Rhesus D antigen
rhG-CSF	recombinant granulocyte-colony stimulating factor
RP	Responsible Person
RPN	risk priority number
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
SAL	sterility assurance level
SAR	serious adverse reaction
SARE	severe adverse reaction or event
SDS	sodium dodecyl sulphate
SEC	Single European Code
SIG	special interest group

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SoHO V&S	Vigilance and Surveillance of Substances of Human Origin
SOP	standard operating procedure
SP-CTO	Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation
S(P)EAR	serious (product) events and reactions
SSC	spermatogonial stem cell
SVF	stromal vascular fraction
T1DM	Type-1 diabetes mellitus
TAMC	total aerobic microbial count
TBV	total blood volume
TCR	T-cell receptor
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TGF	tumour growth factor/transforming growth factor
TNC	total nucleated cells
TNF	tumour necrosis factor
TPV	total plasma volume
TRALI	transfusion-related acute lung injury
TSB	total aerobic microbial count
TSE	transmissible spongiform encephalopathy
TTS	The Transplantation Society
TYMC	total combined yeasts/moulds count
UCB	umbilical cord blood
UPS	uninterrupted power supply
V&S	vigilance and surveillance
vCJD	variant Creutzfeldt–Jakob disease
VEGF	vascular endothelial growth factor
VISTART	Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation [joint action]
VMP	validation master plan
VOC	volatile organic compound
VRE	Vancomycin-resistant *enterococci*
WHO	World Health Organization
WMDA	World Marrow Donor Association
WNV	West Nile virus

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Appendix 3. Glossary

Acceptance criteria	Requirements needed to meet the relevant quality and safety standards in order to ensure an acceptable final product for human application.
Adipose tissue	Loose connective tissue, composed of adipocytes and stromal vascular fraction, which serves as energy storage and endocrine organ.
Advanced therapy medicinal product	A medicinal product that can be a gene therapy medicinal product, a somatic cell therapy medicinal product, a tissue-engineered product or a combined advanced therapy medicinal product (which is a medicinal product incorporating cells and medical devices or actively implantable medical devices).
Adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells. *See also:* Serious adverse event.
Adverse reaction	Any unintended response, including a communicable disease, in the donor or the recipient that is associated with the procurement or human application of tissues and cells. *See also:* Serious adverse reaction.
Agarose gel electrophoresis	Diagnostic tool to visualise DNA fragments.
Alamar blue	Indicator of cell viability based on resazurin oxidation-reduction.
Allogeneic	Refers to tissues and cells removed from one individual and applied to another of the same species.
Allograft	Tissues or cells transplanted between two genetically different individuals of the same species. The term is synonymous with 'homograft'.
AM	Amniotic membrane.
Ambient temperature	The temperature of the surrounding environment. In temperature-controlled facilities, ambient temperature is usually 17-21 °C for thermal comfort. Referred to as 'room temperature' in this Guide.
Amniotic membrane	The innermost layer of the placental membrane; it surrounds the fetus during pregnancy.
Angiogenesis	Physiological process by which new blood vessels form from pre-existing vessels.
Antibiogram	*See:* Resistogram.
Apheresis	A medical technique in which peripheral blood of a donor or patient is passed through an apparatus that separates one particular constituent and returns the remaining constituents to the donor or patient.
Aseptic techniques	Procedures designed to prevent contamination from micro-organisms and spread of infection.
Assisted reproductive technology	All treatments or procedures that include the *in vitro* handling of human oocytes, spermatozoa or embryos for establishing a pregnancy. This includes, but is not limited to, intra-uterine insemination, *in vitro* fertilisation, intracytoplasmic sperm injection, embryo transfer, gamete, germinal tissue and embryo cryopreservation, oocyte and embryo donation and gestational surrogacy.
Audit	Periodic, independent and documented examination and verification of activities, records, processes and other elements of a quality system to determine their conformity with specific internal or external requirements. They may be conducted by professional peers, internal quality system auditors or auditors from certification or accreditation bodies.
Autologous	Refers to tissues or cells removed from and applied in the same individual.
Azoospermia	Absence of spermatozoa in the ejaculate.
Bacteraemia	The presence of viable bacteria in the circulating blood.
Banking	Processing, preservation, storage and distribution of tissues and cells for human application or other purposes, including research and training.
Barcode	An optical machine-readable representation of data relating to the object to which it is attached.

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Batch	A defined quantity of starting material, packaging material or product processed in one process (or series of processes) so that it can be considered to be homogeneous.
Bioactivity	The effect of a substance, upon a living organism.
Biobank	A collection of biological material and the associated data and information stored for research purposes. Also known as a bio-repository.
Bioburden	Total number of viable micro-organisms or total microbial count present, on or in tissues or cells or in the environment, usually measured before the application of a decontamination or sterilisation process.
Biochemical cues	Chemical signal that occurs in a biological organism and causes a biological response.
Biocompatibility	Property of a material being compatible with living tissue. Biocompatible materials do not produce a toxic or immunological response when exposed to the body or body fluids.
Biodegradability	Disintegration of materials by biological processes.
Biomechanical cue	Mechanical signal that occurs in a biological organism and causes a biological response.
Bionics	Biologically inspired engineering is the application of biological methods and systems found in nature to the study and design of engineering systems and modern technology.
Biophysical cue	Physical signal that occurs in a biological organism and causes a biological response.
Bioprinting	Combination of cells, growth factors and biomaterials using layer-by-layer deposition to fabricate biomedical parts that maximally imitate natural tissue characteristics, including structures that are later used in medical and tissue engineering fields.
Bisbenzimidine	Organic compound used as a fluorescent stain for DNA in molecular biology applications.
Blastocyst	An embryo, around 5-6 days after fertilisation, with an inner cell mass, outer layer of trophectoderm and a fluid-filled blastocoele cavity.
Blastomere	A cell in a cleavage stage embryo.
Blood groups	ABO or AB0. Both forms are widely used, but this Guide uses O. The O is from German *ohne* ('without') and means the same as 0 ('zero'): these are red blood cells without A or B antigens on the cell surface.
Bone	The hard, rigid, mineralised form of connective tissue constituting most of the skeleton of vertebrates and composed primarily of calcium salts. There are two types of osseous tissue that form bones: cortical bone (the compact bone of the shaft of a bone that surrounds the marrow cavity) and cancellous or trabecular bone (typically occurs at the ends of long bones, proximal to joints and within the interior of vertebrae). Cancellous bone is highly vascular and frequently contains bone marrow.
Bone marrow	Tissue at the centre of large bones. It is the place where new blood cells are produced. Bone marrow contains two types of stem cell: haematopoietic (which can produce blood cells) and stromal (which can produce fat, cartilage and bone).
Cell	The smallest transplantable and functional unit of life.
Cell culture	Growth of cells in a nutrient medium *in vitro*.
Cell migration	Movement of cells in particular directions, often in response to specific external signals, including chemical signals and mechanical signals.
Circulation	Transfer of tissues or cells from a Tissue establishment to another operator for further processing.
Cytotoxicity	Quality of being toxic to cells.
Clean area, clean environment, cleanroom	An area with defined environmental control of particulate and microbial contamination, and constructed and used in such a way as to reduce the introduction, generation and retention of contaminants within the area.
Cleavage stage embryo	Embryo, beginning with the 2-cell stage and up to, but not including, the morula stage.
Clinical evaluation	Clinical follow-up studies for monitoring predefined clinical outcome indicators to evaluate quality, safety and effectiveness/efficacy of tissue or cell product for a defined number of patients.

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Coding	A system for unique identification of tissues and cells for human application, comprising a donation identifier and product identifier for the specific type of tissue or cell.
Collagen	Main structural protein.
Colonisation	The natural, biological presence or spread of micro-organisms.
Compatibility testing	Testing for the presence or absence of recipient antibodies to HLA and to blood group antigens present on the tissues or cells for transplantation.
Competent authority	*See:* Health Authority.
Computerised system	A system including the input of data, electronic processing and the output of information, to be used either for reporting or for automatic control.
Consent to donation	Lawful permission or authorisation for removal of human cells, tissues and organs for transplantation. *See also:* Opt-in donation; Opt-out donation.
Contained laboratory, contained area	According to EU GMP, an area constructed and operated in such a manner (and equipped with appropriate air handling and filtration) as to prevent contamination of the external environment by biological agents from within the area.
Contamination	Accidental inclusion or growth of harmful micro-organisms, such as bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and by-products. Contamination is different from colonisation, which is the natural, biological presence of micro-organisms.
Controlled ovarian stimulation	Pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes.
Cord blood	Blood collected from placental vessels and umbilical cord blood vessels after the umbilical cord is clamped and/or severed as a source of haematopoietic progenitor cells.
Cord blood bank	A specific type of tissue establishment in which haematopoietic progenitor cells collected from placental and umbilical cord blood vessels are processed, cryopreserved and stored. It may also be responsible for collection, testing or distribution.
Cornea	The transparent anterior part of the outer fibrous coat of the eye. A collagenous tissue bounded by an outer stratified epithelium and an inner monolayer of endothelial cells. The major refractive component of the eye.
Critical	Potentially having an effect on the quality and/or safety of (or having contact with) tissues and cells.
Cross-contamination	Unintentional transfer of micro-organisms and/or other material from one donation or processing batch to another.
Cryopreservation	Preservation and storage of viable tissues and cells (including gametes and embryos) to preserve viability, either by slow freezing or by vitrification.
Cryoprotectant	A chemical compound that is used to protect cells and tissues against freezing injury.
Cumulus cell	The multi-layered mass of granulosa cells surrounding the oocyte.
DAPI	4',6-diamidino-2-phenylindole, a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA.
Deceased donor	A person declared to be dead according to established medical criteria and from whom cells, tissues or organs have been recovered for the purpose of human application. *See also:* Donor after brain death; Donor after circulatory death.
Decontamination	The process of removing or neutralising contaminants.
De-epidermisation	Process by which epidermis is removed from skin.
Delivery rate	Number of deliveries of neonates expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles. It includes deliveries that resulted in the birth of one or more live and/or stillborn babies.
Denudation	The removal or stripping of the cumulus cells from the oocyte.
Design qualification	The first step in the qualification of new equipment or facilities.
Deviation	Departure from an approved instruction/protocol or established standard.
Differentiation	Process by which a less specialised cell becomes a more specialised cell type.
Direct use	Any procedure in which tissues and cells are donated and used without banking or storage.
Discontinuous gradient centrifugation	Sperm-preparation technique based on sedimentation of sperm at different rates depending on density.

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Disinfection	A process that reduces the number of viable micro-organisms, but does not necessarily destroy all microbial forms, such as spores and viruses.
Disposal (of tissues/cells)	The act or means of discarding tissues and/or cells.
Distribution	Transportation and delivery of cells or tissues intended for human application.
Donor	An individual, living or deceased, who is a source of tissues or cells for human application and for other purposes including research.
Donor after brain death	A donor who is declared dead based on the irreversible loss of neurological functions. Also known as deceased heart-beating donor.
Donor after circulatory death	A donor who is declared dead based on circulatory criteria. Also known as deceased non-heart-beating donor.
Donor evaluation	The procedure for determining the suitability of an individual, living or deceased, as a donor of cells or tissues.
Donor selection	*See:* Donor evaluation.
Double embryo transfer	Transfer of two embryos.
Effectiveness	Presence of functionality proven by *in vitro* analytics (e.g. potency assays) depending on the mode of action of the tissue or cell product.
Efficacy	Presence of desired clinical effects / patient outcome depending on the mode of action of the tissue or cell product.
Elastin	Highly elastic protein in connective tissue that allows many tissues in the body to resume their shape after stretching or contracting.
Electron beam irradiation	Use of beta irradiation, usually of high energy under elevated temperatures and nitrogen atmosphere, for sterilisation or cross-linking of polymers.
Embryo	The result of continued development of the zygote to 8 completed weeks after fertilisation, equivalent to10 weeks of gestational age.
Embryo biopsy	The removal of cells (blastomeres or trophectoderm cells) from the embryo for the purpose of genetic analysis.
Embryo donation	Transfer of an embryo resulting from gametes (spermatozoa and oocytes) that did not originate from the recipient and her partner.
Embryo transfer	Procedure in which one or more embryos are placed in the uterus or Fallopian tube.
Emerging disease	A disease that has recently appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range.
End user	A healthcare practitioner who undertakes human application procedures.
Endotoxins	Large molecules consisting of a lipid and a polysaccharide, which are found in the outer membrane of Gram-negative bacteria.
Error	A mistake or failure to carry out a planned action as intended, or application of an incorrect plan that may or may not cause harm to patients.
Escharectomy	Surgical procedure based on removal of necrotic skin tissue from a full-thickness burn.
Ethylene oxide	Organic compound and toxic gas that leaves no residue, being a surface disinfectant widely used in hospitals and the medical equipment industry for sterilisation.
Exceptional release	The distribution for clinical use of a unit of tissues and/or cells that does not fully comply with the defined safety and quality criteria for release. The release is justified by a specific clinical need in which the benefit outweighs the risk associated with the non-compliance. *See also:* Negative-to-date release.
Expert	Individual with the appropriate qualifications and experience to provide technical advice to a health authority inspector.
Expiry date	The date after which tissues or cells are no longer suitable for use. Also known as ‘expiration date’.
Export	Act of transporting a tissue or cell intended for human application to another country where it is to be processed further or used directly. In the EU, ‘export’ refers to transport to a third country (i.e. outside the EU).
Facility	A physical building or part of a building.
Fallopian tube	A long duct in the female abdomen that transports the oocytes that have been released from the ovary to the uterus.

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Fascia	A layer of fibrous connective tissue that surrounds muscles, groups of muscles, blood vessels and nerves; it binds some structures together while permitting others to slide smoothly over each other.
Fertilisation	Entry of the oocyte by a spermatozoon followed by combination of their genetic material, resulting in the formation of a zygote.
Fertility	The capacity to establish a clinical pregnancy.
Fertility preservation	Cryopreservation of reproductive tissues or cells to preserve reproductive capacity.
Fibronectin	High-molecular glycoprotein of the ECM that plays a major role in cell adhesion, growth, migration and differentiation.
Final product	Any tissue or cell preparation intended to be transplanted or administered after the final release step.
Follow-up	Subsequent evaluation of the health of a patient, living donor or recipient, for the purpose of monitoring the results of the donation or human application, maintaining care and initiating post-donation or post-application interventions.
Freeze drying	See: Lyophilisation.
Freezing	As used in this guide, it means storage of tissues at high sub-zero temperatures, with or without cryoprotectant.
Full-thickness grafts (FTSG)	Graft composed of epidermis and full-thickness dermis (with adnexal structure).
Fungaemia	The presence of fungi in the circulating blood.
Gamma irradiation	Penetrating electromagnetic radiation arising from the radioactive decay of atomic nuclei. It is used for medical equipment sterilisation.
Glycosaminoglycans (GAG)	Long unbranched polysaccharides that are highly polar and thus attract water; useful as a lubricant or shock absorber.
Good laboratory practice	Set of principles that provides a framework within which studies are planned, carried out, monitored, recorded, reported and archived by laboratories conducting testing of all kinds.
Good Manufacturing Practice	An EU standard applied internationally for the safe manufacture of medicinal products. Although the processing of tissues and cells is not normally regulated under medicinal manufacturing legislation, many of the principles of GMP can be applied usefully to tissues and cells for human application.
Good practice	A method or technique that has consistently shown results superior to those achieved by other means and which is currently used as a benchmark.
Graft	Part of the human body that is transplanted in the same or another person to replace a damaged part or to compensate for a defect.
Grafting	*See:* Transplantation.
Haematopoietic progenitor cells	Primitive haematopoietic cells capable of self-renewal as well as maturation into any of the haematopoietic lineages, including committed and lineage-restricted progenitor cells, unless otherwise specified and regardless of tissue source. Also referred to as 'haematopoietic stem cells'.
Haematoxylin-Eosin	Histology staining used in medical diagnosis.
Haemodilution	In reference to blood samples from a donor, a decrease in the concentration of the donor's plasma proteins, circulating antigens or antibodies and any infectious agent present, resulting from the transfusion of blood or blood components and/or infusion of fluids, e.g., colloid(s) and/or crystalloid(s). Also known as 'plasma dilution'.
Haemolysis	Damage to red cells resulting in the release of haemoglobin into serum/plasma.
Health Authority	In the context of this Guide, the body which has been delegated with the responsibility for ensuring that tissue and cell donation, banking and human application are appropriately promoted, regulated and monitored in the interests of donor and patient safety and public transparency on a national or regional basis by their government. Other terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it.
Heart valve	One of the four structures within the heart that prevent the backflow of blood by opening and closing with each heartbeat. They include two semilunar valves (aortic and pulmonary), the mitral (or bicuspid) valve and the tricuspid valve. They permit blood flow in only one direction.
Homograft	*See:* Allograft.

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Human application	Use of tissues or cells on or in a human recipient.
Human error	A mistake made by a person rather than being caused by a poorly designed process or by the malfunctioning of a machine such as a computer.
Human tissues and cells for human application	Material containing or consisting of human tissues and/or cells intended for implantation, transplantation, infusion or transfer into or onto a human recipient.
Hybrid scaffold	Scaffold obtained using different types of materials.
Hydroxiproline quantification	Hydroxiproline is a non-essential amino acid (proline derivative) which results from collagen acid hydrolysis.
Identification of tissues and cells	The labelling of tissues and cells to uniquely designate their origin, use or destination. *See also:* Labelling.
Immune-privileged niche	Certain site of the hyman body able to tolerate the introduction of antigens without eliciting an inflammatory immune response.
Implantation (in the context of assisted reproductive technologies)	Attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) that starts 5-7 days after fertilisation. *See also:* Transplantation.
Import	In this context, the act of bringing tissues or cells into one country from another for the purpose of human application or further processing.
Importing tissue establishment	A tissue bank or a unit of a hospital or another body established within the EU which is a party to a contractual agreement with a third-country supplier for the import into the EU of tissues and cells coming from a third country and intended for human application.
Imputability	Assessment of the probability that a reaction in a donor or recipient may be attributable to the process of donation or clinical application or to an aspect of the safety or quality of the tissues or cells applied.
In-process control	Checks undertaken during processing to monitor and, if necessary, to adjust the process to ensure that a product conforms to its specification. Control of the environment or equipment may also be regarded as a part of in-process control.
In vitro fertilisation	Assisted reproductive technology procedure that involves extracorporeal fertilisation. It includes conventional *in vitro* insemination and ICSI, for which see Intracytoplasmic sperm injection.
In vitro maturation	Refers to the maturation of immature oocytes after recovery from follicles that may or may not have been exposed to exogenous gonadotrophins before retrieval. Also, the *in vitro* process of maturation from immature dendritic cells (DC) to mature DC.
Incident	A generic term for an adverse reaction or adverse event.
Incident reporting (adverse event reporting, serious/critical incident reporting)	A system in a healthcare organisation for collecting, reporting and documenting adverse occurrences that affect patients and are inconsistent with planned care (e.g. medication errors, equipment failures, violations).
Informed consent	A person's voluntary agreement, based upon adequate knowledge and understanding of relevant information, to donate, to participate in research or to undergo a diagnostic, therapeutic or preventive procedure.
Inner cell mass	A group of cells in the blastocyst that give rise to the embryonic structures, the fetus, the yolk sac, the allantois and the amnion.
Inspection	On-site assessment of compliance with local/national regulations on tissues and cells, carried out by officials of the relevant Health Authority.
Installation qualification	The second step in the qualification of new equipment or facilities.
Intracytoplasmic sperm injection	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
Intra-uterine insemination	Procedure in which processed sperm cells are transferred transcervically into the uterine cavity.
Keratoplasty	Corneal transplantation.
Key performance indicator	A quantifiable measure or a set of quantifiable measures used to trace performance over time.

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Labelling	Includes steps taken to identify packaged material by attaching the appropriate information to the container or package so it is clearly visible on or through the immediate carton, receptacle or packaging. *See also:* identification of tissues and cells.
Laminine	High-molecular weight protein of the extracellular matrix with important roles in cell differentiation, migration and adhesion.
Laparoscopy	A surgical procedure in which a small incision is made through which a viewing tube (laparoscope) is inserted.
Limbal stem cells	The population of stem cells residing in the basal epithelium of the limbus, giving rise to the corneal epithelium.
Limbal tissue	Tissue bridging the junction between the cornea and sclera. Site of the limbal stem cells that renew the corneal epithelium. Limbal stem cell deficiency causes ocular surface disease.
Limbus	The area bridging the junction between the cornea and sclera.
Live birth rate	Delivery of one or more infants with any signs of life expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles.
Living donor	A living person from whom cells or tissues have been removed for the purpose of human application.
Lyophilisation	A controlled freezing and dehydration process through the sublimation of water under vacuum from ice directly to vapour to a residual water content of $< 5\%$. Typically used to preserve a non-viable perishable material or to make the material more convenient for transport. Also known as freeze drying.
Malignancy	Presence of cancerous cells or tumours with a tendency to metastasise, potentially resulting in death.
Manipulation	Preparation of retrieved tissues or cells to make them suitable for human application. In the context of processing of haematopoietic progenitor cells, this is a laboratory procedure that selectively removes, enriches, expands or functionally alters the cells.
Masson trichrome	Staining protocol used in histology used for distinguishing cells from the surrounding connective tissue.
Medically assisted reproduction (MAR)	Reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, ART procedures, and intra-uterine, intracervical and intravaginal insemination with semen of husband/partner or donor (definition from the International Committee for Monitoring Assisted Reproductive Technology and the World Health Organization, revised glossary of ART terminology, 2009).
Medicinal product	Any substance or combination of substances presented as having properties for treating or preventing disease in human beings, or which may be used in or administered to human beings with a view to either making a medical diagnosis, or restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action.
Meiotic spindle	Spindle apparatus composed of microtubules that support and segregate chromosomes during meiotic division.
Metaphase II oocyte	Mature oocyte at the metaphase of the second meiotic division.
MHC II antigen	Class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells and B cells.
Micromanipulation in ART	Technology that allows micro-operative procedures to be done on the spermatozoon, oocyte, zygote or pre-implantation embryo.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is a colourimetric assay for assessing cell metabolic activity.
Musculoskeletal	Tissues that are part of the skeleton and muscular system, including muscles, bones, cartilage, tendons and ligaments, which function in the support and movement of the body.
Negative-to-date release	The release of tissues or cells for human application before completion of testing for bacterial or fungal cultures. The cultures are negative at the time of release.
Next of kin	A person's closest living blood relative or relatives.
Non-compliance	Failure to comply with accepted standards, requirements, rules or laws.

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Non-partner donation	Donation of reproductive cells between a man and a woman who do not have an intimate physical relationship; also called ‘third-party donation’.
Novelty	Any new tissue and cell preparation or change that could significantly affect the quality and/or safety of tissues and cells and/or the safety of recipients.
Oligozoospermia	Total concentration of spermatozoa $< 15 \times 10^6$/mL.
One-off import	Import of any specific type of tissue or cell that is for the personal use of an intended recipient or recipients known to both the importing tissue establishment and the third-country supplier before the importation occurs. Such an import of any specific type of tissue or cell shall not normally occur more than once for any given recipient. Imports from the same third-country supplier taking place on a regular or repeated basis shall not be considered to be ‘one-off imports’ (Directive 2015/566/EC).
Oocyte	The female gamete (egg).
Oocyte cumulus complex	Oocyte surrounded by the granulosa and corona radiate cells.
Oocyte sharing	Refers to a female partner who enters ART treatment and decides to donate a specified number of her retrieved oocytes. Also known as egg sharing.
Operational qualification	Third step in the qualification of new equipment or facilities.
Opt-in donation	System for determining voluntary consent to donate where consent has been given by an individual during their lifetime or by an individual’s family after their death. Also known as explicit or express consent.
Opt-out donation	System for determining voluntary consent to donate where donation may proceed unless an individual has expressed an objection during their lifetime. Also known as presumed or deemed consent.
Organ	Differentiated and vital part of the human body, formed by different tissues, that maintains its structure, vascularisation and capacity to develop physiological functions with a significant level of autonomy.
Organ culture	Culture of the whole or parts of an organ in medium *in vitro* to preserve cell–cell and cell–matrix interactions and to maintain structure and function.
Organisation responsible for human application	A healthcare establishment or unit of a hospital or another body that carries out human application of human tissues or cells.
Ovarian hyperstimulation syndrome	An exaggerated systemic response to ovarian stimulation characterised by a wide spectrum of clinical and laboratory manifestations. It is classified as ‘mild’, ‘moderate’ or ‘severe’ according to the degree of abdominal distension, ovarian enlargement and respiratory, haemodynamic and metabolic complications.
Package insert	A document included in the packaging of a distributed tissue or cell product that includes important information for the end users on handling, storage, traceability and adverse outcome reporting and, in some cases, on the product’s properties or characteristics.
Packaging	Packaging, including primary and secondary packaging, aims to protect tissues and cells and to present them to the operator (initial or in-process packaging) or to the clinical user (final packaging) in a suitable manner. *See also:* Primary packaging; Secondary packaging.
Packaging material	Any material employed in the packaging of tissues or cells, excluding any outer packaging used for transportation or shipment. Packaging materials are referred to as ‘primary’ or ‘secondary’ according to whether or not they are intended to be in direct contact with the product.
Paracetic acid	Organic compound used for the disinfection of medical supplies to prevent biofilm formation.
Partner donation	Donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship.
Percutaneous epididymal sperm aspiration	Sperm aspiration by percutaneous puncture of the epididymis by a fine-needle technique.
Performance qualification	The fourth step in the qualification of new equipment or facilities.
Pericardium	A double-walled sac that contains the heart and the roots of the great vessels.

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PicoGreen assay	Selective dsDNA quantification of as little as 25 pg/mL.
Placenta	An organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination and gas exchange via the mother's blood supply.
Plasma dilution	*See:* Haemodilution.
Polar body	A haploid cell with very little cytoplasm that is formed and is separated from the oocyte during meiosis and that contains a nucleus produced in the first or second meiotic division.
Pooling	Physical contact or mixing in a single container, of tissues or cells from more than one procurement from the same donor, or from two or more donors.
Posthumous donation	The donation of tissue or cells after the donor's death with prior written consent of the donor.
Pre-implantation genetic testing	A test performed to analyse the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities. These include: PGT for aneuploidies (PGT-A); PGT for monogenic/single gene defects (PGT-M); and PGT for chromosomal structural rearrangements (PGT-SR).
Preservation	Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of tissues or cells.
Primary packaging	Any material employed in the packaging of tissues and cells that is intended to be in direct contact with the graft, excluding any outer packaging used for transportation or shipment.
Procedures	Description of all the tasks, operations and processes to be carried out, the precautions to be taken and measures to be applied to ensure the quality and safety of tissues and cells from procurement through processing, testing and storage to human application.
Processing	All operations involved in the preparation, manipulation, preservation, storage and packaging of tissues or cells intended for human application.
Procurement	A process by which tissues or cells are made available for banking or human application. This process includes donor identification, evaluation, obtaining consent for donation, donor maintenance and retrieval of tissues, cells or organs.
Procurement organisation	A healthcare establishment or a unit of a hospital or another body that undertakes the procurement of human tissues or cells.
Proliferation	Rapid reproduction of a cell.
Pronucleus	The nucleus of the sperm or the oocytes during the process of fertilisation, after the sperm has entered the oocytes but before they fuse.
Prophase I oocyte	Immature oocyte at the prophase of the first meiotic division.
Propidium Iodide	Fluorescent intercalating agent used to evaluate cell viability or DNA content in cell cycle analysis.
Pyrogenic	Producing or produced by heat or fever.
Pyrogens	Substance, typically produced by a bacterium, which produces fever when introduced or released into the blood.
Qualification	According to EU GMP, the action of proving that any equipment works correctly and actually leads to the expected results. More generally, qualification is applied to the inputs to a process, i.e. equipment, facilities, materials and software (and their suppliers), as well as to operators and the relevant written procedures.
Quality	Fulfilment of a specific set of standards, characteristics and requirements.
Quality assurance	The actions planned and performed to provide confidence that all systems and elements that influence the quality of the product are working as expected, both individually and collectively.
Quality control	The part of quality management focused on fulfilling quality requirements. In terms of preparation, it concerns sampling specifications and testing; for an organisation, it relates to documentation and release procedures, which together ensure that the necessary and relevant tests have actually been carried out and that materials have not been released for use until their quality has been judged to be satisfactory.
Quality improvement	The actions planned and performed to develop a system to review and improve the quality of a product or process.

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Quality management system	The organisational structure, with defined responsibilities, procedures, processes and resources, for implementing quality management, including all activities that contribute to quality, directly or indirectly.
Quarantine	The initial status of procured tissues or cells while awaiting a decision on their acceptance or rejection, or tissues or cells isolated physically or by other effective means from other donated material for other reasons until their suitability for use is established.
RABS	Restricted Access Barrier System
Randomised control trial	A study in which samples or subjects are allocated at random into groups, called the 'study' and 'control' groups, to receive or not receive an experimental therapeutic intervention.
Rapid alert	An urgent communication to relevant individuals/organisations to ensure the protection of donors or recipients when an unexpected risk has been identified.
Recall	Removal from use of specific stored or distributed tissues and cells that are suspected or known to be potentially harmful. *See also:* Return; Withdrawal.
Recipient	Person to whom human tissues, cells or reproductive cells and embryos are applied.
Recovery	*See:* Procurement.
Registry	A repository of data collected on tissue, cell and organ donors and/or recipients for the purpose of audit, clinical outcome assessment, quality assurance, validation, healthcare organisation and planning, research and surveillance.
Regulatory authority	*See:* Health Authority.
Release	The act of certifying compliance of a specific tissue or cells of batch of tissues or cells with the requirements and specifications.
Remodelling	Change of the micrometric structure.
Reproductive cells	Oocytes and spermatozoa – in this Guide, oocytes and spermatozoa collected to be used for the purpose of assisted reproduction or fertility preservation.
Resistogram	The result of a test for the sensitivity of an isolated bacterial strain to different antibiotics. Also known as an antibiogram.
Retrieval	*See:* Procurement.
Return	Sending back recalled tissues or cells to the tissue establishment that supplied them for human application.
Risk assessment	Identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm should it occur.
Root cause analysis	A structured approach to investigating and identifying the factors that resulted in the nature, magnitude, location and timing of a harmful or potentially harmful outcome.
Roughness	Quality or state of having an uneven or irregular surface.
Scaffold	A structure made using scaffolding.
Sclera	Fibrous white outer coat of the eye.
Secondary packaging	Any material employed in the packaging of tissues and cells that is not intended to be in direct contact with the graft, and excluding any outer packaging used for transportation or shipment.
Semen analysis	A description of the ejaculate to assess function of the male reproductive tract. Characteristic parameters may include volume and pH, the concentration, motility, vitality and morphology of spermatozoa, and the presence of other cells.
Septicaemia	A systemic disease caused by the spread of pathogenic micro-organisms or their toxins via the circulating blood.
Serious adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or to life-threatening, disabling or incapacitating conditions for a patient, or which might result in, or prolong, hospitalisation or morbidity (Directive 2004/23/EC).
Serious adverse reaction	An unintended response, including a communicable disease, in the donor or in the recipient, associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling or incapacitating or which results in, or prolongs, hospitalisation or morbidity (Directive 2004/23/EC).

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Shipment	A type of transport where the transfer of tissues or cells from the distributing to the receiving facilities is carried out by means of a contract with a third party, usually a specialised logistics company.
Skin	Thin layer of tissue forming the natural outer covering of the human body. Skin is composed of two primary layers: the epidermis and dermis. These layers are separated by a thin sheet of fibres, the basement membrane. Keratinocytes constitute 95 % of the epidermis. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils and elastic fibres, embedded in proteoglycans.
Somatic cells	Any cell of a living organism other than the reproductive.
Spermatozoon	The mature male reproductive cell.
Split-thickness grafts (STSG)	Grafts composed of epidermis and partial-thickness dermis.
Sporicidal	Refers to a substance, agent or product used for killing bacterial spores.
Standard operating procedure	Written instructions describing the steps in a specific process, including the materials and methods to be used and the expected result. *See also:* procedures.
Sterilisation	Any process that eliminates or inactivates transmissible infectious agents (pathogens) containing nucleic acids, e.g. vegetative and spore forms of bacteria and fungi, parasites or viruses, present on a surface, in a fluid, in medication or in a compound such as biological culture media. Sterilisation can be achieved by applying the proper combinations or conditions of heat, chemicals, irradiation, high pressure and filtration.
Sterility assurance level	Represents the expected probability of a micro-organism surviving on an individual unit of product after exposure to a sterilisation process. SAL 10^{-6} has been established as the standard for allografts and indicates a probability of one chance in a million that one unit of product will be contaminated with a single organism after a sterilisation process. If the product meets or or exceeds this standard, grafts are then considered sterile.
Storage	Maintenance of a product under appropriate controlled conditions until distribution.
Storage temperature	Temperature at which tissues and cells must be stored to maintain their required properties.
Supercritical carbon dioxide	Fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure. It is an alternative for terminal sterilisation of biological materials and medical devices with combination of the paracetic acid.
Supernumerary embryos	Excess embryos after embryo transfer.
Surveillance	Systematic collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health responses, as necessary.
Swim up	A preparation technique based on the ability of spermatozoa to swim in the culture medium.
Tendon	A tough band of fibrous connective tissue that usually connects muscle to bone and which can withstand tension.
Terminal sterilisation	A method for achieving the sterility of a product in its sealed container and with a sterility assurance level of 10^{-6} or better.
Testicular sperm extraction/aspiration	A surgical procedure involving testicular biopsies or needle aspirations to obtain sperm for use in IVF and/or ICSI.
Third countries	Term used within the EU to refer to countries that are not members of the EU.
Third party	Any organisation that provides a service to a procurement organisation or tissue establishment on the basis of a contact or written agreement.
Time-lapse imaging	The photographic recording of microscope image sequences. In this Guide, used for documentation of gametes, zygotes, cleavage-stage embryos or blastocysts at regular intervals.
Tissue	An aggregate of cells joined together by, for example, connective structures and performing a particular function.
Tissue bank	*See:* Tissue establishment.
Tissue establishment	A facility or a unit of a hospital or another organisation where the activities of processing, preservation, storage or distribution of human tissues and cells for

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	human application are undertaken. It may also be responsible for procurement and/or testing of tissues and cells.
Toxicity	Degree to which a substance can damage an organism.
Traceability	Ability to locate and identify a specific tissue/cell during any step from procurement, through processing, testing and storage, to distribution to the recipient or disposal. This implies the ability to identify: the donor; the tissue establishment or processing facility that receives, processes or stores the tissue and cells; and the recipient(s) at the medical facility/facilities applying the tissues and cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues and cells.
Transmissible disease	Comprises all clinically evident illnesses (i.e. characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of micro-organisms in an individual or the transmission of genetic conditions to the offspring. In the context of transplantation, malignancies and autoimmune diseases may also be transmitted from donor to recipient.
Transplantation, implantation or grafting	Transfer (engraftment) of human tissues or cells from a donor to a recipient with the aim of restoring function(s) in the body. *See also:* Implantation (in the context of assisted reproductive technologies).
Transport	The act of transferring a tissue or cellular product between distributing or receiving facilities under the control of trained personnel.
Trophectoderm	Outer layer of cells in a blastocyst (composed of trophectoderm and inner cell mass cells). A group of cells in the blastocyst that do not produce any embryonic structures but give rise to the chorion, the embryonic portion of the placenta.
Unique identification code	A code that unambiguously identifies a particular donor and donation (e.g. a unique donation + tissue product code). *See also:* Coding.
Validation	Documented evidence giving a high degree of assurance that a specific process or system, including pieces of equipment or the environmental conditions, will perform consistently to deliver a product meeting its pre-determined specifications and quality attributes, based on intended use.
Vas deferens, vasa deferentia	Tube(s) that transport(s) sperm from the epididymis to the ejaculatory ducts.
Verification	Preferred term for the validation or qualification of IT systems/software.
Vigilance	Alertness to and/or awareness of serious adverse events, serious adverse reactions or complications related to donation and human application of tissues, cells and organs, involving an established process for reporting at local, regional, national or international level. *See also:* Surveillance.
Viraemia	The presence of viruses in the blood.
Vitrification	Method of ice-free cryopreservation achieved through an extreme elevation in solution viscosity sufficient to suppress the crystallisation of water. Requires rapid cooling and/or high concentrations of solutes, such as the conventional cryoprotectants, to reach the glass transition temperature without ice formation.
Wettability	Tendency of one fluid to spread on, or adhere to, a solid surface in the presence of other immiscible fluids. Wettability refers to the interaction between fluid and solid phases.
Window period	Period of time before infection can be detected by a specific testing method.
Withdrawal	Process instigated by a tissue establishment to recall tissues or cells that have been distributed.
Xenograft	Graft of tissue taken from a donor of one species and grafted into a recipient of another species.
Xenotransplantation	Any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live tissues, cells or organs from a non-human animal source, or (b) human body fluids, tissues, cells or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs.
Zygote	A diploid cell resulting from the fertilisation of an oocyte by a spermatozoon, before completion of the first mitotic division.

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Appendix 4. Example of cleanroom qualification

Validation Protocol

Validation Title	Qualification of a Clean room	CC/

Short description of equipment or process being validated.

Qualification of clean rooms for use in regulated environments

Details of equipment used in the validation.

An active Environmental Monitoring System (EMS)

Settle plates

Contact / air sampling plates

Particle counter

Active air sampler

Details of testing levels and methods used in validation

Eudralex Volume 4 "The rules governing medicinal products in the European Union", Annex 1 Manufacture of sterile medicinal products

SOP254 – Environmental monitoring using contact plates

SOP975 – Environmental monitoring using the active air sampling

SOP978 – Environmental monitoring using settle plates

SOP2382 – Environmental monitoring equipment

SOP4007 – SCI clean room cleaning

ISO14644 – BSEN14644 and EU GMP clean room standards

INSTALLATION QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Clean room designed in accordance to required operating specifications	Appropriate specifications available and clean room designed to meet specifications. Current drawings for clean room layout and air handling unit(s) are available.				
2.	Clean room layout, fixtures and finishes are installed according to the current drawings and are of an appropriate standard.	Clean room finishes are smooth, impervious, non-shedding and crack and crevice free. Floor to wall, wall to wall and wall to ceiling junctions are coved and finished in vinyl and are defect free. All wall and ceiling penetrations are fully sealed with silicone sealant and are defect free. Light fittings and filter housings are surface mounted and are fully sealed with silicone sealant and are defect free. There are no un-cleanable recesses and minimal projecting ledges, shelves, cupboards and equipment. Fixtures, fittings and clean room furniture are all present, secure and free of rust and defects. Clean room entry / exit doors and pass-through hatch doors are interlocked or otherwise controlled to prevent both doors being opened simultaneously.				
3.	Confirm access to the EMS system data is available	Records must be accessible during the validation process				

4.	Confirm that particle counters and differential pressure monitoring systems are calibrated and available	In date calibration certificates must be available and equipment free for use during the entire validation period				
5.	Ensure clean room and associated air handling unit(s) is registered as an asset in QPulse	QPulse asset number must be generated				

OPERATIONAL QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Particle Challenge Leak Test for testing of each installed HEPA filter using DOP	External contractor to perform DOP testing of facilities in accordance with ISO 14644-3. Aerosol concentrations must be ≤ 0.01% of the upstream concentration				
2.	Air exchange rate testing. Tested in accordance to BSEN 14644-3-2005	External contractor to measure airflow volume or airflow velocity. Air change rate in compliance with design specification and should achieve > 20 Air changes per hour				
3.	Particle Counting for classification of the clean room	External contractor to perform particle counting in clean room to meet EU GMP Annex 1 “at rest” limits for particulates (working to ISO 14644-1)				
4.	Air flow distribution testing using smoke visualisation	External contractor to perform smoke visualisation test in accordance with ISO14644-3 demonstrating: • Flow distribution is satisfactory within each room • Any dead spots within each room have been identified • No areas of excessive turbulence below working height exist (that could lead to particulate contamination)				
5.	Perform a weekly clean of the clean room as per SOP4007	Clean room cleaning must be easy to facilitate and unobstructed				
6.	Perform weekly at rest environmental monitoring as per SOP254, SOP975 and SOP978.	Full set of plates must be exposed and results shown to not exceed EU GMP Annex 1 limits for microbial contamination.				
7.	Perform routine weekly at rest particle counting monitoring as per SOP2382.	Full set of counts must be obtained in accordance with SOP2382, and checked for compliance with EU GMP Annex 1 “at rest” limits.				

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
8.	Record the differential pressures for clean room facilities during “at rest” monitoring.	Daily records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades at rest).				
9.	Perform simulated operation environmental monitoring as per SOP254, SOP975 and SOP978.	Full set of plates must be exposed whilst non-clinical clean room work is performed, and results shown not to exceed EU GMP Annex 1 limits for microbial contamination.				
10.	Perform simulated operation particle counting monitoring.	Full set of counts must be obtained whilst non-clinical clean room work is performed and checked to ensure compliance with EU GMP Annex 1 “in operation” limits.				
11.	Record the differential pressures for clean room facilities “simulated operation” monitoring.	Records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades during working).				
12.	Review at rest and simulated operation results and data.	Results should not highlight any problems or trends. All results must not exceed the upper limits for the relevant grades of room, in compliance with EU GMP Annex 1				
13.	Particulate clean-up rate within stipulated limits.	Particulate air recovery/clean up rate must be achieved within 20 minutes in the at rest state and after operators/working has left the room (and after simulated operation tests).				

PERFORMANCE QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Perform a weekly clean of the clean room as per SOP4007 for a minimum of an 8 week period.	Clean room cleaning must be easy to facilitate and unobstructed.				
2.	Perform environmental monitoring as per SOP254, SOP975 and SOP978 for a minimum of 8 consecutive weeks.	Full set of plates must be exposed at least weekly (in either the at rest or in use state) and results shown not to exceed EU GMP Annex 1 limits.				
3.	Perform particle counting monitoring for a minimum of 8 consecutive weeks.	Full set of counts must be obtained at least once per week and in accordance with SOP2382, and checked for compliance with EU GMP Annex 1 limits (at rest or in operation, as appropriate for time of monitoring).				
4.	Record the differential pressures for clean room facilities for a minimum of 8 weeks of continued monitoring.	Records must be obtained for differential pressures for each day that environmental monitoring is performed, and checked for compliance with the design specification and EU GMP (10-15 Pa between adjacent rooms of different grades.				
5.	Review results and data. Identify any issues and trends	Results should not highlight any problems or trends. All results must not exceed the action limits for the relevant grades of room, in compliance with EU GMP Annex 1.				

NOTE: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live

Deviations and Adverse Events

QPulse no.	Details	Date raised	Date closed

FURTHER TESTING DETAILS: (If applicable)

Appendix 5. Example of incubator qualification

Validation Protocol

Validation Title	Clean room CO_2 Incubators	CC/

Short description of equipment or process being validated.

Clean room incubators are used within processing to incubate samples at a set temperature for culture. Temperature of each incubator needs to be assessed prior to use in order to evaluate suitability of incubator for use and position of the temperature monitoring system probe.

Details of equipment used in the validation.

Calibrated temperature monitoring devices.

Supplier:____ ______________________

Model:______ ____________________

Serial No. _____ ____________________

Details of testing levels and methods used in validation

See IQ, OQ, PQ description and acceptance criteria.

Temperature mapping carried out as per SOP XXX

Recorded on FRM XXX

INSTALLATION QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Site incubator	Undamaged on delivery and fits designated area satisfactorily.				
2.	Ensure that cleanroom air flow is not affected	Air flow is satisfactory				
3.	Instruction manual	Manual present				
4.	Certificate of conformance	Certificate of conformance				
5.	Register warranty	Register warranty				
6.	Add to asset register	Add to asset register				
7.	Cleaning instructions provided by supplier	Instructions supplied				

OPERATIONAL QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Incubator functions	Switches on				
2.	Ensure shelves fitted correctly	Shelves fitted correctly				
3.	Create SOP and FRM for Incubator use, cleaning and maintanence	FRM and SOP created				
4.	Clean incubator as per instructions provided	Batch numbers/expiry of cleaning products recorded				
5.	Swab each shelf onto TSA and SABC agar plates and send for incubation	Swab results clear and appended				
6	Set temperature to required level	Set temperature to required setting.	Temperature setting: °C			
7	Set CO_2 % level to required level	CO_2 level set to required setting	CO_2 level setting: %			

PERFORMANCE QUALIFICATION

Validation Title	Clean room CO2 Incubators	CC/

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Perform initial temperature mapping (EMPTY)	Satisfactory as per SOP XXX				
2.	Site temperature mapping probe	As informed by step 1				
3.	Connected to Environmental monitoring system (EMS) and ensure temperature alarms are set	Connected for both high and low alarms Low Alarm Limit: High Alarm limit: Delay Time:	EMS Alarm name: Low Alarm Limit: High Alarm limit:		Append EMS record	
4.	Set CO_2 levels on Environmental monitoring system (EMS)	Connected for both high and low alarms. Delay time for alarms calculated by comparing readings on incubator with EMS. Low Alarm Limit: High Alarm limit: Delay Time:	EMS Alarm name: Low Alarm Limit: High Alarm limit: Delay time:		Append EMS record	
5.	Perform empty but humidified temperature mapping	Satisfactory as per SOP XXX				
6.	Perform simulated / full load, humidified temperature mapping	Satisfactory as per SOP XXX			Simulated load details recorded in mapping record	
7.	Enable EMS	EMS alarms enabled				

NOTE: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live

Deviations and Adverse Events

QPulse no.	Details	Date raised	Date closed

FURTHER TESTING DETAILS: (If applicable)

Appendix 6. Example of validation of a tissue

TRANSPORTATION METHOD

Tissue transportation*

The example of a process validation outlined below describes a process that will be common to most, if not all, tissue establishments – that is, the need to transport tissues from one place to another (for example, from the site of procurement to the processing facility, or from the tissue bank to the end user). Control of the conditions of transportation is critical for ensuring tissue quality. The example below refers specifically to the transport of skin allografts from the procurement site to a tissue establishment at refrigerated temperatures. However, the principles are identical for all types of transportation.

The first stage is to define the process in detail. This is achieved by addressing the following questions:

a. Which type of tissue, and what maximum volume of it, will be transported?
b. How is the tissue contained? What is the nature, volume and temperature of any transport solution to be used? Which type of packaging has been used?
c. Which refrigerant has been used and what is its specification and volume?
d. What are the specifications of the transport container (dimensions, insulation etc.)?
e. What are the most extreme transportation conditions allowable in terms of transport time and ambient temperature?

Once the process has been defined, the acceptance criteria need to be defined. In our example, the criteria were that:

- the temperature of the skin allograft must remain at 0-10 °C for the duration of the transit;
- the integrity of the tissue packaging must be maintained during transit;
- the integrity of the transport container must be maintained during transit;
- the pH of the transportation fluid must be 7.0-7.5 at the end of the transportation.

For some tissues, it may be advisable to go further and validate the quality of the tissue after transit (e.g. assessment of its viability or histological structure).

In our example it was determined that the maximum amount of skin to be transported would be 6 000 cm^2, immersed in a minimum volume of 300 mL of transport fluid. Specifications of the packaging, transport container and refrigerant were also documented. The most extreme acceptable transport conditions were defined as an ambient temperature of 40 °C (e.g., a hot summer day in a vehicle) for a maximum of 12 h, with the minimum volume of refrigerant and transport solution and the maximum volume of tissue.

A protocol was written and a model prepared using skin obtained from donors unsuitable for clinical donation. This protocol was based on the defined transport solution, refrigerant, packaging and container specifications. A calibrated data-logging thermometer was used to record the temperature on the external surface of the tissue packaging. The container was placed into a shaking incubator set at an ambient temperature of 40 °C. A shaking incubator was used to model the agitation of the container during vehicular transit (the model should approximate as closely as practically possible real-life conditions).

The study was repeated in triplicate. Acceptable results were obtained on each occasion. All results were well within the pre-defined acceptance criteria, so the process was accepted based on the results of the three replicates.

---

* Reproduced with permission from: Winters M, Lomas R. The principles of process validation and equipment qualification. In: Fehily D, Brubaker S, Kearney J, Wolfinbarger L, editors. *Tissue and cells processing: an essential guide*. London, UK: Wiley-Blackwell; 2012.

Note that it may be necessary to find a compromise between an ‘ideal’ validation and operational practicalities that cannot be avoided. For example, it may not be possible or ethical to obtain and sacrifice large amounts of tissue for validation studies. In these cases, an acceptable compromise should be reached using risk-assessment principles (e.g. use of animal tissue as a substitute).

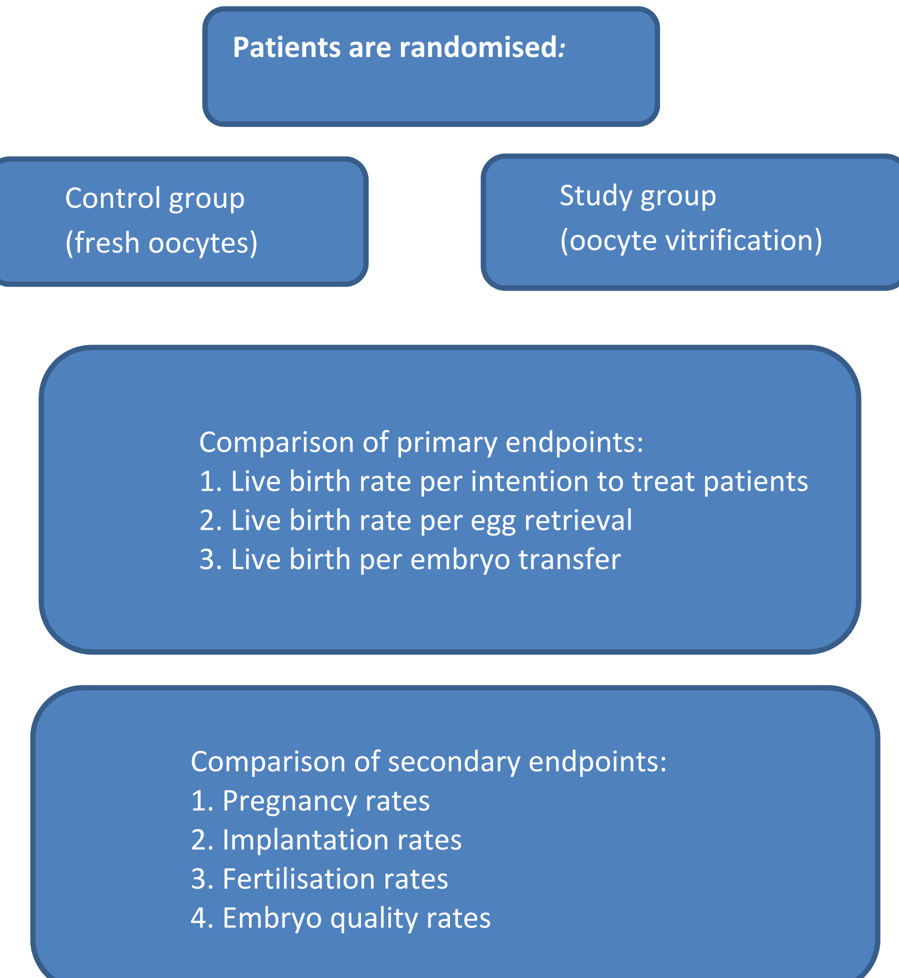
Application of sufficiently robust process validations (e.g. by challenging a transport process with extremes of time and temperature) obviates the need for routine temperature monitoring of the process. Thus, if the physical conditions identified by the validation study are complied with (e.g. the correct container, containing at least the minimum amount of refrigerant, in transit for less than the maximum modelled time), then it can be reliably concluded that the process itself has been carried out correctly. Therefore, to demonstrate compliance with the validated process, all operatives need to do is to confirm that they have complied with the relevant standard operating procedures.

Appendix 7. Method validation- oocyte vitrification

Oocyte vitrification

If and when a validation is performed, a randomised clinical study with defined endpoints (primary and secondary) should be defined and, if it is a multicentre study, agreed upon between the assisted reproductive technologies (ART) centres. See Figure 7.A.

Figure 7.A. Randomised clinical study



However, for a single ART centre it may be difficult to perform a randomised study, because of the number of patients that must be included to override biological variations. As an alternative, a randomised controlled trial can be used to validate the standard method in parallel with the new method to be validated, following the layout below. See Figure 7.B.

Figure 7.B. Randomised controlled trial

Oocytes (from the same patient) are randomised:

Control group
(fresh oocytes)

Study group
(oocyte vitrification)

Comparison of primary endpoints:

1. Fertilisation rates per utilised oocyte

2. Embryo quality rates per utilised oocyte

Comparison of secondary endpoints:

1. Implantation rates
2. Pregnancy rates
3. Live birth rates

If and when a validation is performed in a randomised clinical study with defined endpoints (see Fig. 7A), usually a multi-centre study is needed and the endpoints (primary and secondary) should be defined and agreed upon between the ART centres. See Figure 7.C.

Figure 7.C. Multi-centre randomised control trial

Patients are randomised*:*

Control group
(fresh oocytes)

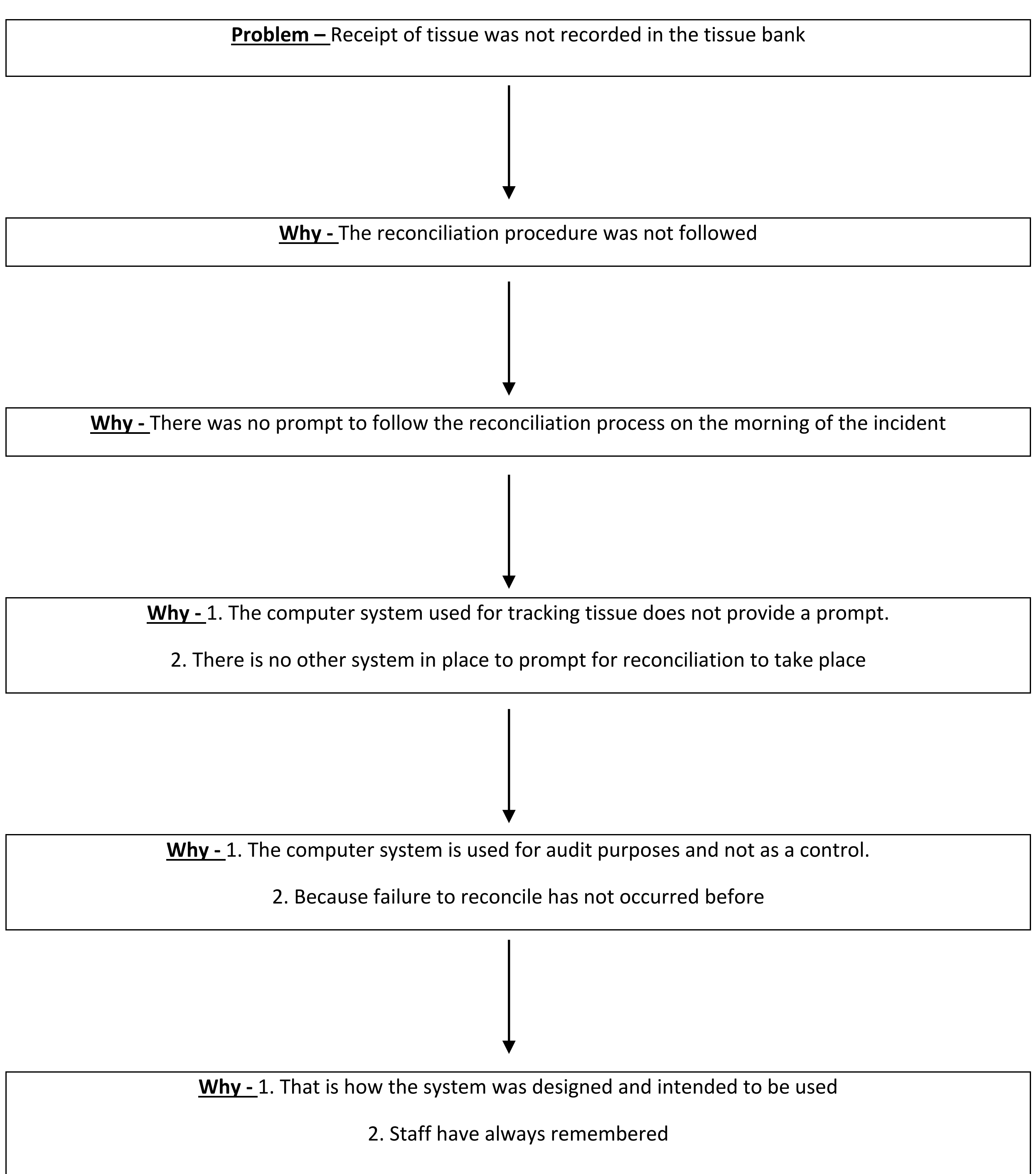
Study group
(oocyte vitrification)

Comparison of primary endpoints:
1. Live birth rate per intention to treat patients
2. Live birth rate per egg retrieval
3. Live birth per embryo transfer

Comparison of secondary endpoints:
1. Pregnancy rates
2. Implantation rates
3. Fertilisation rates
4. Embryo quality rates

Appendix 8. Example of root cause analysis- why, why?

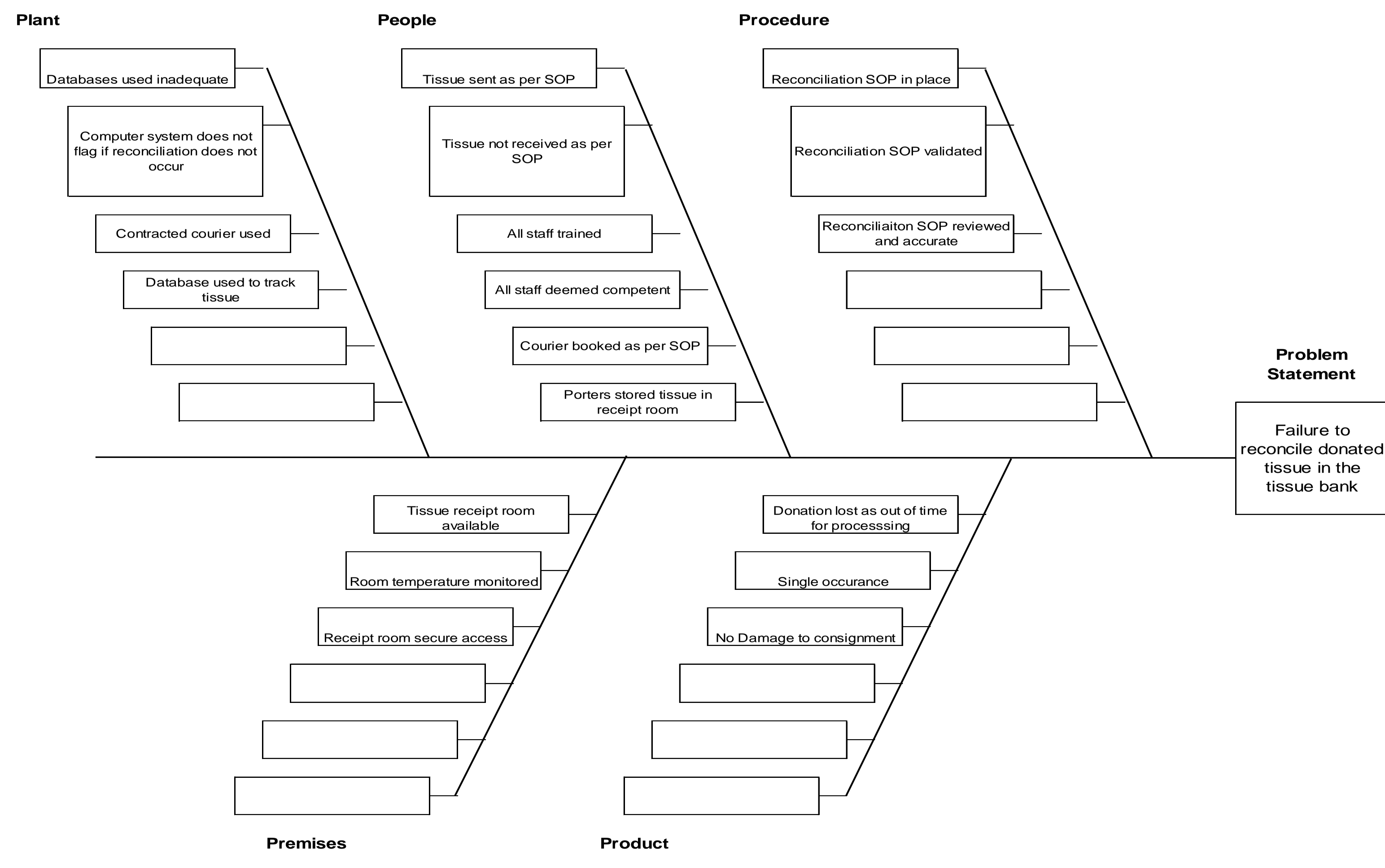
Why Why Example



Root Cause

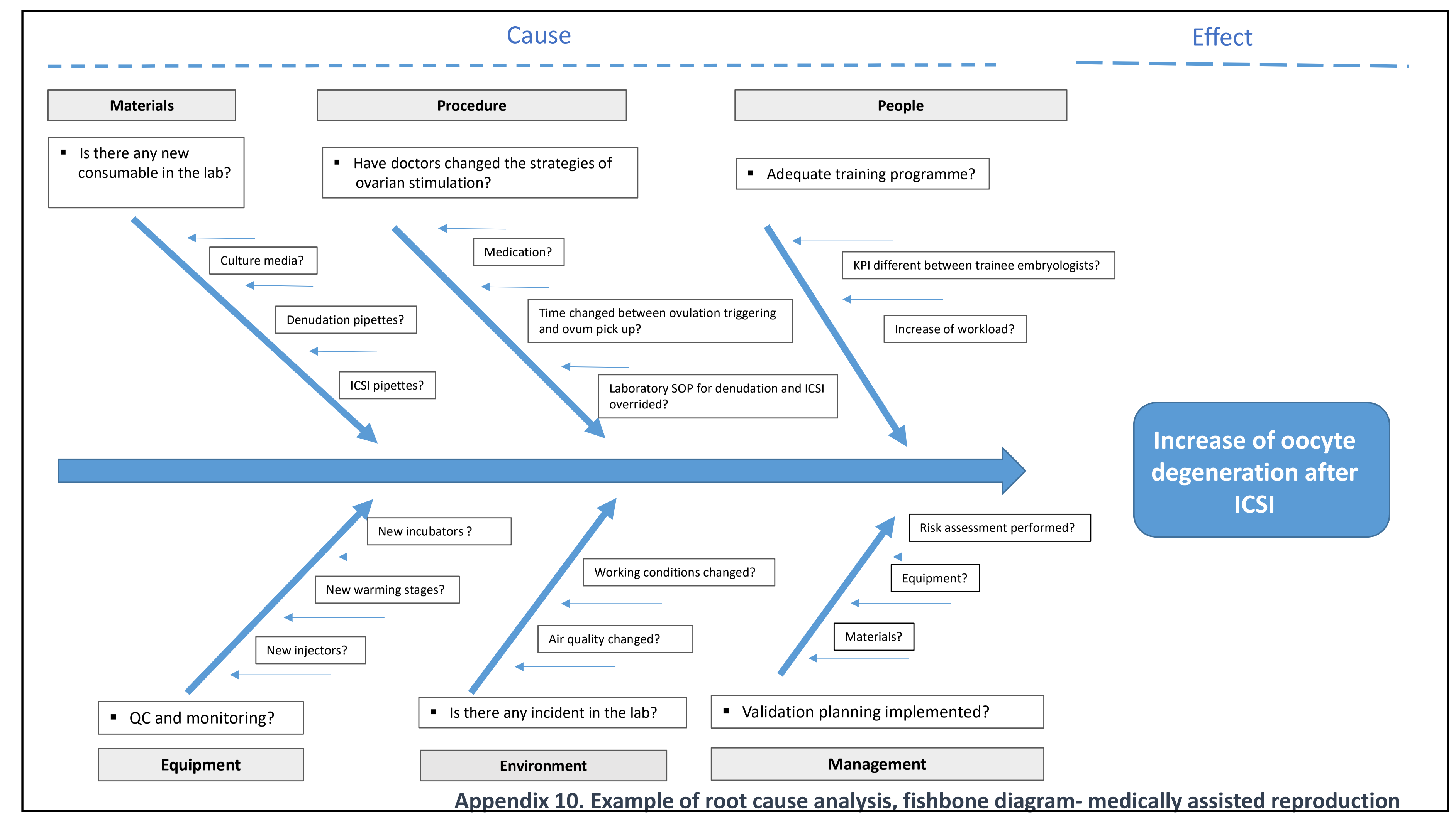
There is a reliance on staff to reconcile. There is no system in place to prompt for reconciliation to take place.

Appendix 09. Fishbone-edGMFH



Root Causes:
1. Reconciliation procedure not followed by tissue bank staff following the receipt and storage of tissue by the porters
2. Computer system does not alert if receconciliation is not compelted

Appendix 10. Sample Consent form



Appendix 10. Example of root cause analysis, fishbone diagram- medically assisted reproduction

Women's consent to treatment and storage form (IVF and ICSI)

HFEA WT form

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about us, visit **www.hfea.gov.uk**.

Who should fill in this form?

Fill in this form if you are a woman and you are having fertility treatment using embryos created outside the body (in vitro) with your eggs. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
  - the different options set out in this form
  - the implications of giving your consent
  - the consequences of withdrawing this consent, and
  - how you can make changes to, or withdraw, your consent.
- an opportunity to have counselling.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. If you do not receive this information before filling in this form, your consent may be invalid.

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your eggs, and embryos created using your eggs, to be used or stored (eg, for IVF or ICSI treatment). If you are storing your eggs or embryos, you must also state in writing how long you consent to them remaining in storage.

You are also legally required to record what you would like to happen to your eggs and embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, your clinic needs to know this so that they only allow your eggs and embryos to be used according to your wishes. If you are unsure of anything in relation to this, please ask your clinic.

Why are there questions about using my eggs and embryos for training purposes?

You may have some eggs and embryos left after treatment which you do not wish to use (eg, because you do not want future treatment or the eggs and embryos are not viable for treatment). On this form, you can consent to donate these for training purposes to allow healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

What if I want to donate my eggs and/or embryos?

Unused eggs and embryos can also be donated for research purposes, helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Your clinic can give you more information about this and provide you with the relevant consent form(s).

You could also think about donating viable unused eggs and embryos to another person for use in their treatment. Before doing this, there are lots of issues to consider. For more information, see **www.hfea.gov.uk/egg-and-sperm-donors.html**. If you decide to donate, you will need to complete a separate form: 'Your consent to donating your eggs' (WD form).

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the consent and information given. When you have completed the form you may request a copy of it from your clinic.

For clinic use only (optional)

HFEA centre reference ☐☐☐☐

Other relevant forms ☐

Date embryos were placed in storage D D M M Y Y

Date embryos can remain in storage until D D M M Y Y

1 About you

1.1 **Your first name(s)**

Place clinic sticker here

1.2 **Your surname**

1.3 **Your date of birth**

D D M M Y Y

1.4 **Your NHS/CHI/HCN/passport number** (please circle)

2 About your partner

Place clinic sticker here

2.1 **Your partner's first name(s)**

2.2 **Your partner's surname**

2.3 **Your partner's date of birth**

D D M M Y Y

2.4 **Your partner's NHS/CHI/HCN/passport number** (please circle)

3 Your treatment

3.1 **Do you consent to your eggs being used to create embryos outside the body for your treatment (eg, through IVF treatment)?**

In order to create embryos for your treatment you must provide your consent by ticking the yes box below. Please note that the sperm provider also has to give his consent for embryos to be created.

☐ Yes

4 Storing embryos

4.1 **Do you consent to the embryos (created outside the body with your eggs) being stored?**

Please note that embryos can only be stored if the sperm provider has also given his consent.

☐ Yes ▸ after signing the page declaration below, continue on the next page.

☐ No ▸▸ now sign the page declarations on this page and the next page then go straight to section five.

▸▸▸▸ Continues on the next page

Page declaration

Your signature

✗

Date

D D M M Y Y

4 Storing embryos *continued*

Embryo storage periods

You may wish to store any embryos left after treatment so they can be used in future treatment. To be stored, embryos are frozen or 'vitrified'. When considering how long to store for, you may want to think about how far in the future you might want/be able to use your stored embryos and the costs of storing – ask your clinic if you are unsure. The law permits you to store for any period up to 10 years but in cases where you or your partner are prematurely infertile, or likely to become prematurely infertile, you may store for longer, up to 55 years.

Please note that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store your embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

4.2 Have you, or your partner, been diagnosed as prematurely infertile or likely to become prematurely infertile?

Causes of premature infertility can include chemotherapy treatment and early menopause. Please speak to your clinic if you are unsure. If your circumstances change and either you or your partner become prematurely infertile, or are likely to become prematurely infertile, you and your partner can change your consent to store your embryos for up to 55 years.

☐ No ▸ go to 4.3.

☐ Yes ▸▸ go straight to 4.4.

4.3 For how long do you consent to store your embryos?

You can consent to store your embryos for up to 10 years. Please note that the sperm provider also has to give his consent to storage.

☐ For 10 years

☐ For a specific period (up to a maximum of 10 years) ▸ specify the number of years:

☐ years

The consent period will start from the date of storage. Remember you can always change the time period you consent to by completing this form again and specifying the new total time period you would like your embryos to be stored for. For example, if you consented to five years' storage on the original form and wish to consent for a further five years (10 years in total), you should complete another copy of this form but tick the box for 10 years. This second form would supersede the first form you completed. ▸▸ **Now sign the page declaration below and go straight to section five.**

4.4 Premature infertility

If you or your partner are prematurely infertile, or likely to become prematurely infertile, you can consent to store your embryos for up to 55 years. Although you can consent up to a maximum of 55 years on this form, after the first 10 years your medical practitioner will need to certify in writing that the medical criteria for premature infertility have been met for storage to continue for more than 10 years.

▸▸▸▸ Continues on the next page

Page declaration

Your signature

✗

Date

D D M M Y Y

For clinic use only (optional) Patient number

4 Storing embryos *continued*

When the criteria have been met, the storage period will be extended by 10 years from the date the criteria are met. The storage period can then be extended by further 10 year periods (up to a maximum of 55 years) at any time within each extended storage period if it is shown that the criteria continue to be met. For more information about this, please ask your clinic.

For how long do you consent to store your embryos?

Please specify the number of years you consent to store your embryos for (up to a maximum of 55): ☐ years.

Clinic staff: please attach all relevant medical practitioners' statements to this form.

5 Using eggs and embryos for training

5.1 **Do you consent to your eggs being used for training purposes?**

☐ Yes ☐ No

5.2 **Do you consent to embryos (already created outside the body with your eggs) being used for training purposes?**

Please note that embryos can only be used if the sperm provider has also given his consent.

☐ Yes ☐ No

6 In the event of your death or mental incapacity

As part of your consent, you also need to decide what you would like to happen to your eggs, or embryos created outside the body with your eggs, if you die or lose the ability to decide for yourself (become mentally incapacitated). Please note your embryos may only be used within the storage period you consented to above. **If you do not give your consent in the below section, your eggs or embryos must be allowed to perish in the event of your death or mental incapacity and cannot be used for treatment.**

6.1 **Do you consent to your eggs being used for training purposes?**

If you die	If you become mentally incapacitated
☐ Yes ☐ No	☐ Yes ☐ No

6.2 **Do you consent to embryos (already created outside the body with your eggs) being used for training purposes?**

Please note that embryos can only be used if the sperm provider has also given his consent.

If you die	If you become mentally incapacitated
☐ Yes ☐ No	☐ Yes ☐ No

Other uses for your eggs or embryos

If you wish your eggs or embryos to be used in someone else's treatment if you die or become mentally incapacitated, please speak to your clinic for more information. Depending on your circumstances, you will need to complete one of the following: • 'Your consent to donating your eggs' (WD form), • 'Your consent to donating embryos' (ED form), or • 'Women's consent to the use and storage of eggs or embryos for surrogacy' (WSG form).

Page declaration

Your signature

✗

Date

D D M M Y Y

For clinic use only (optional) Patient number ☐

7 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
  - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling
  - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me, and
  - I understand that I can make changes to, or withdraw, my consent at any point until the time of embryo transfer, use of eggs or embryos in training, or the eggs or embryos have been allowed to perish.
- I declare that the information I have given on this form is correct and complete.
- I understand that information on this form may be processed and shared for the purposes of, and in connection with, the conduct of licensable activities under the Human Fertilisation and Embryology Act 1990 (as amended) in accordance with the provisions of that act.

Your signature

✗

Date

D D M M Y Y

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because she is unable to sign for herself due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with her direction.

Representative's name

Representative's signature

✗

Relationship to the person consenting

Date

D D M M Y Y

Witness's name

Witness's signature

✗

Date

D D M M Y Y

D

For clinic use only (optional) Patient number

CRYOPRESERVATION OF OOCYTES AND/OR OVARIAN TISSUE

Informed Consent

Oocytes are female reproductive cells that, in their immature form, are already present in the ovaries at the moment of birth. From puberty onwards, during each normal menstrual cycle, groups of oocytes undergo maturation phenomena that results in the release by the ovary of an oocyte that is mature and can therefore be fertilised.

In certain clinical situations - when essential treatments threaten the survival of reproductive cells, for example cryopreservation of oocytes has been proposed in an attempt to protect future fertility.

The cryopreservation of oocytes may also be justified in other clinical situations.

A number of key points should be highlighted:

- Cryopreservation of oocytes is a technique the global implications of which are not yet a matter of consensus in the scientific world.
- Cryopreservation of oocytes does not guarantee that a pregnancy will be obtained; it only guarantees a reserve of female reproductive cells that can be used in future. Currently, the rate of pregnancy achieved with *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) of cryopreserved oocytes is lower.
- Given the small number of children born as a result of the use of cryopreserved oocytes there is no reliable information as regards a possible increased risk of foetal abnormalities.
- Cryopreservation of oocytes does not establish any obligation on this centre in relation to the future use of those cells, nor does it grant the person from whom they originated any type of preferential treatment; at all times, the clinical criteria of good clinical practice appropriate to each situation will be applied.
- You alone, and no one else, have the right to use the cryopreserved oocytes.
- Unforeseen accidents, such as fires or calamities of other types, may, despite the safety precautions taken, lead to loss or destruction of the cryopreserved oocytes.

CONSENT

I, the undersigned, declare that:

- I have read and understood this document and the additional information provided.
- The queries and questions I have raised have been answered.
- I recognise that this text cannot describe exhaustively all the situations that could arise in the future.
- I have understood and I accept that the oocytes and/or ovarian tissue will be cryopreserved for a maximum period of five years and that I may revoke this authorisation at any time during this period.
- I am aware that I alone have the right to use these frozen oocytes and/or ovarian tissue.
- I have understood that, under the prevailing legislation, at the end of this five-year period, I will have to travel to the centre to sign a consent form for this freezing to continue. In the absence of a signed declaration requesting a further period of cryopreservation, I declare that I have been clearly informed that the oocytes and/or ovarian tissue will be thawed and destroyed, unless I hereby express authorisation for their use for scientific purposes. In those circumstances (write **Yes** or **No**):

  - I consent to use of my oocytes in scientific research projects ☐
- I fully understand and accept the conditions, risks and limitations set out above.

Therefore, having been fully informed, I freely assume the obligations arising from conclusion of this agreement and give my consent for the use of this technique in preserving my oocytes.

NAME ____

SIGNATURE ____

CIVIL ID/PASSPORT NO. ____

Clinician: ____ ____/____/ _____

Men's consent to treatment and storage form (IVF and ICSI)

HFEA MT form

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about us, visit **www.hfea.gov.uk**.

Who should fill in this form?

Fill in this form if you are a man and your partner is having fertility treatment using embryos created outside the body (in vitro) with your sperm. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
  - the different options set out in this form
  - the implications of giving your consent
  - the consequences of withdrawing this consent, and
  - how you can make changes to, or withdraw, your consent.
- an opportunity to have counselling.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. If you do not receive this information before filling in this form, your consent may be invalid.

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you. However, if you are consenting to being registered as the legal father of any child born as a result of treatment after your death (see section 6.5), you **must** sign the form yourself.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your sperm, and embryos created using your sperm, to be used or stored (eg, for IVF or ICSI treatment). If you are storing your sperm or embryos, you must also state in writing how long you consent to them remaining in storage.

You are also legally required to record what you would like to happen to your sperm and embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, your clinic needs to know this so that they only allow your sperm and embryos to be used according to your wishes. If you are unsure of anything in relation to this, please ask your clinic.

Why are there questions about using my sperm and embryos for training purposes?

You may have some sperm and embryos left after treatment which you do not wish to use (eg, because you do not want future treatment or the sperm and embryos are not viable for treatment). On this form, you can consent to donate these for training purposes to allow healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

What if I want to donate my sperm and/or embryos?

Unused sperm and embryos can also be donated for research purposes, helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Your clinic can give you more information about this and provide you with the relevant consent form(s).

You could also think about donating viable unused sperm and embryos to another person for use in their treatment. Before doing this, there are lots of issues to consider. For more information, see **www.hfea.gov.uk/egg-and-sperm-donors.html**. If you decide to donate, you will need to complete a separate form: 'Your consent to donating your sperm' (MD form).

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the consent and information given. When you have completed the form you may request a copy of it from your clinic.

For clinic use only (optional)

HFEA centre reference

Other relevant forms

Date embryos were placed in storage

D D M M Y Y

Date embryos can remain in storage until

D D M M Y Y

1 About you

1.1 **Your first name(s)** *Place clinic sticker here*

1.2 **Your surname**

1.3 **Your date of birth**

D D M M Y Y

1.4 **Your NHS/CHI/HCN/passport number** (please circle)

2 About your partner

2.1 **Your partner's first name(s)** *Place clinic sticker here*

2.2 **Your partner's surname**

2.3 **Your partner's date of birth**

D D M M Y Y

2.4 **Your partner's NHS/CHI/HCN/passport number** (please circle)

3 Your treatment

3.1 **Do you consent to your sperm being used to create embryos outside the body for your partner's treatment (eg, through IVF treatment)?**

In order to create embryos for your partner's treatment you must provide your consent by ticking the yes box below. Please note that the egg provider also has to give her consent for embryos to be created.

☐ Yes

4 Storing embryos

4.1 **Do you consent to the embryos (created outside the body with your sperm) being stored?**

Please note that embryos can only be stored if the egg provider has also given her consent.

☐ Yes ▸ after signing the page declaration below, continue on the next page.

☐ No ▸▸ now sign the page declarations on this page and the next page then go straight to section five.

▸▸▸▸ Continues on the next page

Page declaration

Your signature

✗

Date

D D M M Y Y

4 Storing embryos *continued*

Embryo storage periods

You may wish to store any embryos left after treatment so they can be used in future treatment. To be stored, embryos are frozen or 'vitrified'. When considering how long to store for, you may want to think about how far in the future you might want/be able to use your stored embryos and the costs of storing – ask your clinic if you are unsure. The law permits you to store for any period up to 10 years but in cases where you or your partner are prematurely infertile, or likely to become prematurely infertile, you may store for longer, up to 55 years.

Please note that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store your embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

4.2 Have you, or your partner, been diagnosed as prematurely infertile or likely to become prematurely infertile?

Causes of premature infertility can include chemotherapy treatment and early menopause. Please speak to your clinic if you are unsure. If your circumstances change and either you or your partner become prematurely infertile, or are likely to become prematurely infertile, you and your partner can change your consent to store your embryos for up to 55 years.

☐ No ▸ go to 4.3.

☐ Yes ▸▸ go straight to 4.4.

4.3 For how long do you consent to store your embryos?

You can consent to store your embryos for up to 10 years. Please note that the egg provider also has to give her consent to storage.

☐ For 10 years

☐ For a specific period (up to a maximum of 10 years) ▸ specify the number of years:

☐ years

The consent period will start from the date of storage. Remember you can always change the time period you consent to by completing this form again and specifying the new total time period you would like your embryos to be stored for. For example, if you consented to five years' storage on the original form and wish to consent for a further five years (10 years in total), you should complete another copy of this form but tick the box for 10 years. This second form would supersede the first form you completed. ▸▸ **Now sign the page declaration below and go straight to section five.**

4.4 Premature infertility

If you or your partner are prematurely infertile, or likely to become prematurely infertile, you can consent to store your embryos for up to 55 years. Although you can consent up to a maximum of 55 years on this form, after the first 10 years your medical practitioner will need to certify in writing that the medical criteria for premature infertility have been met for storage to continue for more than 10 years. When the criteria have been met, the storage period will be extended by 10 years from the date the criteria are met.

▸▸▸▸ Continues on the next page

Page declaration

Your signature ✗

Date D D M M Y Y

4 Storing embryos *continued*

The storage period can then be extended by further 10 year periods (up to a maximum of 55 years) at any time within each extended storage period if it is shown that the criteria continue to be met. For more information about this, please ask your clinic.

For how long do you consent to store your embryos?

Please specify the number of years you consent to store your embryos for (up to a maximum of 55): ☐ years.

Clinic staff: please attach all relevant medical practitioners' statements to this form.

5 Using sperm and embryos for training

5.1 **Do you consent to your sperm being used for training purposes?**

☐ Yes ☐ No

5.2 **Do you consent to embryos (already created outside the body with your sperm) being used for training purposes?**

Please note that embryos can only be used if the egg provider has also given her consent.

☐ Yes ☐ No

6 In the event of your death or mental incapacity

As part of your consent, you also need to decide what you would like to happen to your sperm, or embryos created outside the body with your sperm, if you die or lose the ability to decide for yourself (become mentally incapacitated). Please note that if you would like your partner to use your sperm or embryos in the event of your death or mental incapacity, your partner should be named on this form. Your embryos may only be used within the storage period you consented to above.

If you do not give your consent in the below section, your sperm or embryos must be allowed to perish in the event of your death or mental incapacity and cannot be used for treatment.

6.1 **Do you consent to your sperm being used to create embryos outside the body for your partner's treatment?**

Please note that the egg provider also has to give her consent for embryos to be created.

If you die	If you become mentally incapacitated
☐ Yes ☐ No	☐ Yes ☐ No

6.2 **Do you consent to embryos (already created outside the body with your sperm) being used for your partner's treatment?**

Please note that embryos can only be used if the egg provider has also given her consent.

If you die	If you become mentally incapacitated
☐ Yes ☐ No	☐ Yes ☐ No

▸▸▸▸ Continues on the next page

Page declaration

Your signature

✗

Date

D D M M Y Y

6 In the event of your death or mental incapacity *continued*

6.3 Do you consent to your sperm being used for training purposes?

If you die

☐ Yes ☐ No

If you become mentally incapacitated

☐ Yes ☐ No

6.4 Do you consent to embryos (already created outside the body with your sperm) being used for training purposes?

Please note that embryos can only be used if the egg provider has also given her consent.

If you die

☐ Yes ☐ No

If you become mentally incapacitated

☐ Yes ☐ No

Other uses for your sperm or embryos

If you wish your sperm or embryos to be used in someone else's treatment if you die or become mentally incapacitated, please speak to your clinic for more information. Depending on your circumstances, you will need to complete one of the following:

- 'Your consent to donating your sperm' (MD form)
- 'Your consent to donating embryos' (ED form), or
- 'Men's consent to the use and storage of sperm or embryos for surrogacy' (MSG form).

Consent to birth registration

Complete this part of section six if you consented to your sperm, or embryos created outside the body with your sperm, being used in your partner's treatment after your death.

If you have given your consent to your sperm or embryos (to be created outside the body with your sperm) being used after your death, you may also wish to consent to being registered as the legal father of any child that is born as a result of your partner's treatment.

6.5 Do you consent to being registered as the legal father of any child born as a result of your partner's treatment after your death?

By ticking yes, you consent to the following:

- I consent to my name, place of birth and occupation being entered on the register of births as the legal father of any child born from my partner's treatment.

  This register is kept under the Births and Deaths Registration Act 1953, or the Births and Deaths Registration (Northern Ireland) Order 1976, or the Registration of Births, Deaths and Marriages (Scotland) Act 1965.
- I also consent to information about my or my partner's treatment being disclosed to my partner and one of the following registrars:
  - the Registrar General for England and Wales
  - the Registrar General for Scotland
  - the Registrar for Northern Ireland.

Please note that being recorded in the register of births as the legal father of a child born from your partner's treatment does not transfer any inheritance or other legal rights to the child.

☐ Yes ☐ No

Page declaration

Your signature

✗

Date

D D M M Y Y

For clinic use only (optional) Patient number

7 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
  - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling
  - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me, and
  - I understand that I can make changes to, or withdraw, my consent at any point until the time of embryo transfer, use of sperm or embryos in training, or the sperm or embryos have been allowed to perish.
- I declare that the information I have given on this form is correct and complete.
- I understand that information on this form may be processed and shared for the purposes of, and in connection with, the conduct of licensable activities under the Human Fertilisation and Embryology Act 1990 (as amended) in accordance with the provisions of that act.

Your signature

✗

Date

D D M M Y Y

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because he is unable to sign for himself due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form. However, if the person consenting consented to being registered as the legal father after his death (that is if he ticked yes to question 6.5), he **must** sign the form himself.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with his direction.

Representative's name

Representative's signature

✗

Relationship to the person consenting

Date

D D M M Y Y

Witness's name

Witness's signature

✗

Date

D D M M Y Y

For clinic use only (optional) Patient number

Tissue Donor Number | | | | | | | | |

ODT Donor Number | | | | | |

Medical and Social History Questionnaire

Directions for completion

1 This form must be completed in **black or dark blue ink** by the Specialist Nurse – Organ Donation (SNOD)/Specialist Nurse – Tissue Donation (SNTD)/Tissue Donor Co-ordinator (TDC) and signed where required.

2 The original copy should be retained by the **SNOD/SNTD/TDC** for the donor file.

3 In the event of organ and tissue donation, a legible copy should be sent to the relevant **Tissue Establishment**, where required.

NOTE: The term patient is used throughout the form to refer to the potential donor.

The term relative is used throughout the form to refer to the relationship between the patient and the interviewee.

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

In order to ensure the safety of organs and tissue for transplant I will need to ask you some questions about *(name of patient)* medical and lifestyle history. Some of the questions are of a sensitive and personal nature. They are similar questions to those asked when someone donates blood. I will read and discuss each question with you and ask that you answer to the best of your knowledge with either a “Yes” or “No.”

PATIENT INFORMATION

Patient’s Forename(s) Please print

Patient’s Surname Please print

Donating Hospital

NHS/CHI Number

Cause of Death

Hospital Number

Date of Birth (dd/mm/yyyy)

Occupation

Country of Birth

Country of Residency

INTERVIEWEE INFORMATION

Information discussed with

Name Please print

Relationship Please print

For patients under the age of 18 months, or those who have been breast-fed or fed breast milk by a donor in the last 12 months, the mother is required to answer these questions with regard to her own and her child’s health.

For children: has your child been breast-fed in the past 12 months? Yes ☐ No ☐ Unknown ☐

NOTE: for all patients under the age of 18 months and any child who has been breast-fed in the last 12 months, a blood sample for microbiological testing is required from the mother, as well as from the patient.

For ALL female patients between 13 and 53 years of age: Is there a possibility that your relative could be pregnant? Yes ☐ No ☐ Unknown ☐

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

GENERAL HEALTH INFORMATION

1. Did your relative visit a general practitioner in the last two years? Yes ☐ No ☐ Unknown ☐

If YES, give details

2. Was your relative currently seeing or waiting to see a general practitioner or any other healthcare professional? Yes ☐ No ☐ Unknown ☐

If YES, give details

3. Did your relative ever take regular medication? Yes ☐ No ☐ Unknown ☐

If YES, give details of any current or previous medication including any medication for acne, prostate or psoriasis

4a. Did your relative have a history of allergies to medication, food or other substances? Yes ☐ No ☐ Unknown ☐

If YES, please provide details of the substance they were allergic to and describe the reaction

4b. Did your relative have any health problems due to exposure to toxic substances such as pesticides, lead, mercury, gold, asbestos, cyanide, agent orange etc? Yes ☐ No ☐ Unknown ☐

If YES, please provide details of the toxic substance and treatment

5a) Was your relative a diabetic? Yes ☐ No ☐ Unknown ☐

If YES, were they on insulin? Yes ☐ No ☐ Unknown ☐ N/A ☐

5b) Is there a family history of diabetes? Yes ☐ No ☐ Unknown ☐

If YES, is it insulin-dependent diabetes? Yes ☐ No ☐ Unknown ☐ N/A ☐

6. Did your relative suffer from any chronic or autoimmune illness or disease of unknown cause? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

7. Did your relative ever suffer from any bone, joint, skin or heart disease? Yes ☐ No ☐ Unknown ☐

If YES, specify which and give details

8. Did your relative ever have hepatitis, jaundice or liver disease? Yes ☐ No ☐ Unknown ☐

If YES, give dates, diagnosis, treatment and hospital /clinic name if known

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

GENERAL HEALTH INFORMATION

9. Did your relative recently suffer from significant unplanned weight loss? Yes ☐ No ☐ Unknown ☐

If YES, give details

10. Did your relative ever undergo any investigations for cancer or were they ever diagnosed with cancer? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

11. Did your relative have a history of eye disease, receive any medications for eye problems (e.g. eye drops), or undergo eye surgery or laser treatment? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

12. Did your relative ever have any operations? ***If NO go to question 15*** Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

13. Did your relative ever have any surgery on the brain or spine? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details including hospital name and dates of treatment if possible. **Surgery before 1993** is particularly significant

14. Did your relative ever have an organ or tissue transplant? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details including hospital name and dates of treatment if known

15. Was your relative ever told not to donate blood? Yes ☐ No ☐ Unknown ☐

If YES, give details of where, when and the reason

16. Did your relative receive a transfusion of blood or blood product(s) at any time? Yes ☐ No ☐ Unknown ☐

If YES, give details including country, hospital name, dates and reason for transfusion

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

GENERAL HEALTH INFORMATION

17. Did your relative suffer from any type of brain disease such as Parkinson or Alzheimer disease or dementia? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

18. Did your relative suffer from any one or more of the following problems: memory problems or confusion, change in personality or behaviour, or were they unsteady on their feet? If **NO** go to Question 19, if **YES** Yes ☐ No ☐ Unknown ☐

18a. Were you aware of a condition causing these symptoms? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, please specify condition

18b. When did these symptoms start?

Please give details

18c. Did they worsen noticeably over time?

Please give details

18d. Was your relative able to live independently?

Please give details

19. Did your relative have a family history of prion disease, such as CJD, or were they ever told that they were at risk of prion disease? Yes ☐ No ☐ Unknown ☐

If YES, please give details

20. Did your relative ever receive human pituitary extracts, e.g. growth hormones or fertility treatment or test injections for hormone imbalance? Yes ☐ No ☐ Unknown ☐

If YES, give details including dates and hospital/clinic name if known

21. Did your relative ever have any significant infection? Yes ☐ No ☐ Unknown ☐

If YES, give details, and any treatment received and hospital/clinic name if known

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

RECENT HISTORY

22. Did your relative come into contact with an individual with an infectious disease within the last month? Yes ☐ No ☐ Unknown ☐

If YES, please specify details, dates, symptoms, diagnosis, and treatment

23. Did your relative have any signs of infection, e.g. colds, flu, fever, night sweats, swollen glands, diarrhoea, vomiting or skin rash within the last month? Yes ☐ No ☐ Unknown ☐

If YES, please specify dates, symptoms, diagnosis, and treatment

24. Did your relative have any immunisations within the last 2 months? Yes ☐ No ☐ Unknown ☐

If YES, give details including travel vaccinations and flu vaccination or flu nasal spray

25. Did your relative have tattooing, body piercing, botox injections, acupuncture, colonic irrigation, faecal transplantation, or any other cosmetic treatments or injuries that involve piercing the skin in the last 3 months? Yes ☐ No ☐ Unknown ☐

If YES, give details including where and when including unlicensed clinics in UK or abroad

26. In the last 12 months has your relative been bitten or scratched by any animal (strays, pets, wild, farm or ticks) or been bitten by a human. Or, has your relative ever been bitten or in close contact with bats anywhere in the world or been bitten by a mammal outside the UK? Yes ☐ No ☐ Unknown ☐

If YES, give details of incident, circumstances, animal, place, dates and treatment

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

TRAVEL HISTORY

27. Did your relative ever travel or live outside the UK (including business trips)? Yes ☐ No ☐ Unknown ☐
If NO go to question 33

28. In the last 12 months did your relative go outside the UK (including business trips)? Yes ☐ No ☐ Unknown ☐ N/A ☐

Give details of dates and destinations visited

29. Did your relative ever have malaria or an unexplained fever which they could have picked up whilst abroad? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give date of fever/illness, places visited, duration and dates

30. Was your relative ever unwell whilst abroad or in the first month of their return to the UK? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details

31. Did your relative ever live or travel outside the UK for a continuous period of 6 months or more? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details of dates and destinations

32. Did your relative ever go to Central America, Mexico or South America for a continuous period of 1 month or more? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details of dates, places (remote/rural/urban areas), nature of visit

33. Was your relative's mother born in Central America, Mexico or South America? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

BEHAVIOURAL RISK ASSESSMENT

34. Did your relative
    (a) Consume alcohol? Yes ☐ No ☐ Unknown ☐

If YES, give details

(b) Smoke tobacco or any other substance? Yes ☐ No ☐ Unknown ☐

If YES, give details of substance, frequency, history of smoking time and time elapsed since giving up

(c) Take any recreational drugs? Yes ☐ No ☐ Unknown ☐

If YES, give details of route of administration and dates

35. Is it possible that any of the following apply to your relative?

(a) Was, or may have been infected with HIV, hepatitis or HTLV? Yes ☐ No ☐ Unknown ☐

(b) Within the last 12 months have they injected, or been injected, with non-prescription drugs, including performance enhancing drugs or injectable tanning agents? Yes ☐ No ☐ Unknown ☐

(c) Been in prison or a juvenile detention centre for more than 3 consecutive days in the last 12 months? Yes ☐ No ☐ Unknown ☐

(d) Taken medication to prevent HIV infection e.g. (PrEP Pre/Post exposure prophylaxis)? Yes ☐ No ☐ Unknown ☐

If YES to any of the above questions a-d, give details, including dates for question c

36. Has your relative **ever** had sex – consensual or otherwise?
    If no, go to question 38. Yes ☐ No ☐ Unknown ☐

If **YES**, is it possible that your relative:

(a) Was given payment for sex with money or drugs in the last 3 months? Yes ☐ No ☐ Unknown ☐ N/A ☐

(b) Ever had a sexually transmitted disease? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details, including hospital/clinics, dates, treatments.

Question	Yes	No	Unknown	N/A
37. Did your relative have sex, consensual or otherwise in the last 3 months?	☐	☐	☐	☐
If no, go to question 38. If yes, is it possible that in the last 3 months your relative had sex with:				
(a) *(for male patients only)* another man?	☐	☐	☐	☐
(b) *(for female patients only)* a man who has ever had sex with another man?	☐	☐	☐	☐
(c) Anyone who is HIV or HTLV positive?	☐	☐	☐	☐
(d) Anyone who has hepatitis?	☐	☐	☐	☐
(e) Anyone who had a sexually transmitted disease?	☐	☐	☐	☐
(f) Anyone who has ever been given payment for sex with money or drugs?	☐	☐	☐	☐
(g) Anyone who in the last 12 months has injected or been injected with non-prescription drugs including performance enhancing drugs or injectable tanning agents?	☐	☐	☐	☐

If YES, give details

Question	Yes	No	Unknown	N/A
(h) Anyone who could have had sex, in any part of the world, where AIDS/HIV is very common (this includes most countries in Africa)?	☐	☐	☐	☐
(i) Anyone who has developed an illness related to travel such as Zika?	☐	☐	☐	☐
38. Having answered all the previous questions, is there anyone else who you think may provide more information?	☐	☐		

If YES, please specify

Tissue Donor Number

ODT Donor Number

Question number	Relevant additional information. If any questions have been answered as unknown, give an explanation

Signature of healthcare professional obtaining information

Please print name

Designation of healthcare professional obtaining information

Date of interview 2 0

Time of interview

Appendix 15. Physical Assessment Form (Dutch Transplant Foundation)

Donor identification:
Donor number:
Date of birth: Gender ☐ M ☐ F
Date recovery:

Identification verification: ☐ No ☐ Yes
Consent: ☐ No ☐ Yes

Recovery team members:

Start time recovery

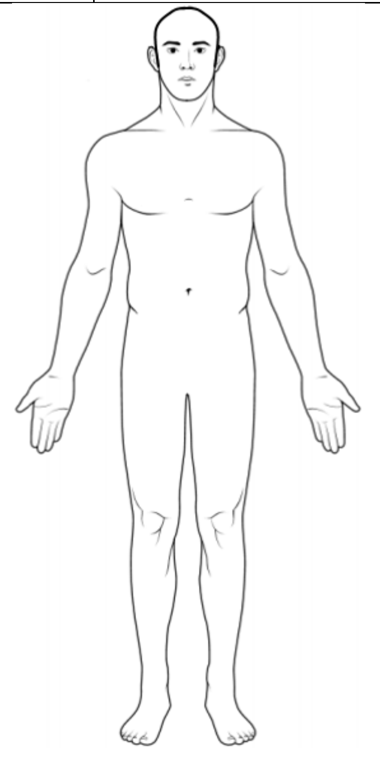
Eye tissue		Skin	
Heart valves		MS tissue	
Thoracic aorta		Femoral arteries	

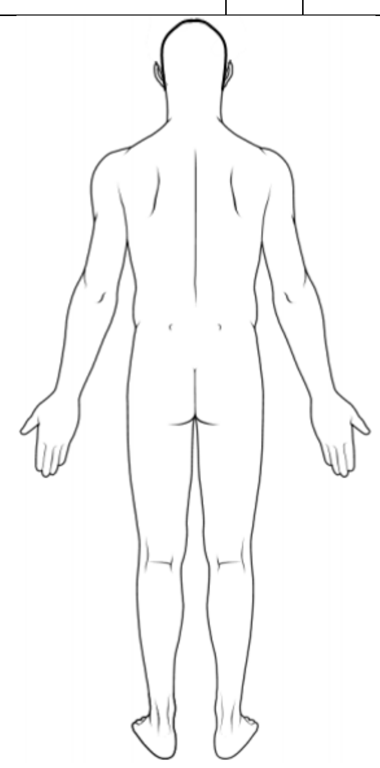
Complications during procedure: ☐ No ☐ Yes

General appearance Good / Moderate / Poor
Height: cm Weight: kgs

(O)	Ocular abnormalities	☐ No	☐ Yes	☐ Unable to visualize	
(WS)	White spots in the mouth	☐ No	☐ Yes	☐ Unable to visualize	
(J)	Jaundice:	☐ No	☐ Yes		
(LN)	Abnormal lymph node(s)	☐ No	☐ Yes	Location? Size? Consistency?	
(L)	Enlarged liver	☐ No	☐ Yes		
(H)	Hematoma / bruises	☐ No	☐ Yes		
(GL/PL)	Genital and/or perianal lesions	☐ No	☐ Yes		
(NMI)	Non-medical injection sites	☐ No	☐ Yes		
(SL)	Skin lesions	☐ No	☐ Yes	Requires description	
(S)	Scars	☐ No	☐ Yes	☐ Old	☐ Recent
(Ta/Pi)	Tattoo / Piercings	☐ No	☐ Yes	☐ Old	☐ Recent

(IV)	IV / Arterial line	(P)	Pacemaker / ICD	(BN)	Bone needle
(MP)	Needle entry site (medical procedures)	(D)	Drainage	(St)	Stoma
(BC)	Needle site blood collection	(C)	Cast	(Ca)	Catheter
(B)	Bandage	(I)	Autopsy/Organ recovery incision	(De)	Decubitus





Describe Findings/Tattoos:

Consultation ☐ No ☐ Yes

Photo's taken? ☐ No ☐ Yes

Notes:

Appendix 16. Evaluation of pigmented skin lesions

A careful physical examination of the donor should be conducted, paying particular attention to the skin, looking for potential neoplasms or scars of previous surgical procedures (see Chapter 4).

The ‘ABCDE rule’ is an easy guide to detecting the usual signs that may be indicative of melanoma [1-2].

	(A) Asymmetry If one half is not identical to the other half, suspect melanoma.
	(B) Border irregularity Notched, scalloped, ragged or poorly defined borders should cause us to suspect melanoma.
	(C) Colour Naevi usually have a uniform colour; if there is colour variability from black-brown to red-blue-grey or white, suspect melanoma.
	(D) Diameter/Difference If diameter is >6 mm, suspect melanoma. Small lesions with some of the previous characteristics should also lead us to suspect melanoma. If there are multiple lesions with a more or less regular aspect, but there is one that has a very ‘ugly’ aspect compared to the rest (ugly ducking sign), suspect melanoma.
	(E) Evolution If there was an evolution or change in appearance of a lesion, suspect melanoma. Any change – in size, shape, colour, elevation or another trait, or any new symptom such as bleeding, itching or crusting – points to danger.

Source of images: www.dermatology.ucsf.edu/skincancer/general/types/melanoma.aspx and www.skincancer.org/skin-cancer-information/melanoma.

Other warning signs are:

- Naevi are the most powerful predictor of risk of melanoma. An individual with more than 100 common naevi or more than two atypical naevi has a 5- to 20-fold increased risk of melanoma;
- People with a first-degree relative with melanoma are at increased risk of developing melanoma; 5-10 % of individuals with melanoma have a family

history of melanoma. If there is a suspicious lesion and there is family history of melanoma, suspect melanoma.

References

1. Friedman RJ, Rigel DS, Kopf AW. Early detection of malignant melanoma: The role of physician examination and self-examination of the skin. *CA Cancer J Clin* 1985;**35**(3):130-51.
2. Whited JD, Grichnik JM. The rational clinical examination. Does this patient have a mole or a melanoma? *JAMA* 1998;**279**(9):696-701.
3. Gachon J, Beaulieu P, Sei JF *et al*. First prospective study of the recognition process of melanoma in dermatological practice. *Arch Dermatol* 2005;**141**(4):434-8.

Appendix 17. Evaluation of malignancies for risk assessment in tissue and cell donors

Table 17.A. WHO classification of myeloid neoplasms and acute leukaemia

Source: Arber DA, Orazi A, Hasserjian R *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;**127**(20):2391-405 and *Blood* 2016;**128**(3):462-3.

Myeloproliferative neoplasms (MPN)

Chronic myeloid leukaemia (CML), *BCR-ABL1*$^{+}$
Chronic neutrophilic leukaemia (CNL)
Polycythemia vera (PV)
Primary myelofibrosis (PMF)
- PMF, prefibrotic/early stage
- PMF, overt fibrotic stage

Essential thrombocythemia (ET)
Chronic eosinophilic leukaemia, not otherwise specified (NOS)
MPN, unclassifiable
Mastocytosis

Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*, or with *PCM1-JAK2*

Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement
Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement
Myeloid/lymphoid neoplasms with *FGFR1* rearrangement
Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

Chronic myelomonocytic leukaemia (CMML)
Atypical chronic myeloid leukaemia (aCML), *BCR-ABL1*$^{-}$
Juvenile myelomonocytic leukaemia (JMML)
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
MDS/MPN, unclassifiable

Myelodysplastic syndromes (MDS)

MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
- MDS-RS and single lineage dysplasia
- MDS-RS and multilineage dysplasia

MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood
Myeloid neoplasms with germ line predisposition

Acute myeloid leukaemia (AML) and related neoplasms

AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);-*CBFB-MYH11*
- APL with *PML-RARA*
- AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
- AML with t(6;9)(p23;q34.1);*DEK-NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);-*RBM15-MKL1*

- *Provisional entity: AML with BCR-ABL1*
- AML with mutated *NPM1*
- AML with biallelic mutations of *CEBPA*
- *Provisional entity: AML with mutated RUNX1*

AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS

- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukaemia
- Acute monoblastic/monocytic leukaemia
- Pure erythroid leukaemia
- Acute megakaryoblastic leukaemia
- Acute basophilic leukaemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma
Myeloid proliferations related to Down syndrome

- Transient abnormal myelopoiesis (TAM)
- Myeloid leukaemia associated with Down syndrome
-

Blastic plasmacytoid dendritic cell neoplasm

Acute leukaemias of ambiguous lineage

Acute undifferentiated leukaemia
Mixed phenotype acute leukaemia (MPAL) with t(9;22)(q34.1;q11.2); *BCR-ABL1*
MPAL with t(v;11q23.3); *KMT2A* rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS

B-lymphoblastic leukaemia/lymphoma

B-lymphoblastic leukaemia/lymphoma, NOS
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
B-lymphoblastic leukaemia/lymphoma with hypodiploidy
B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
Provisional entity: B-lymphoblastic leukaemia/lymphoma, BCR-ABL1–like
Provisional entity: B-lymphoblastic leukaemia/lymphoma with iAMP21

T-lymphoblastic leukaemia/lymphoma

Provisional entity: Early T-cell precursor lymphoblastic leukaemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukaemia/lymphoma

Table 17.B.   WHO classification of lymphoid neoplasms

Source: Swerdlow SH, Campo E, Pileri SA *et al*. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*, 2016 May 19;**127**(20):2375-90.

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
γ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
_Large B-cell lymphoma with IRF4 rearrangement_*
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
_EBV$^+$ mucocutaneous ulcer_*
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK$^+$ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
_HHV8$^+$ DLBCL, NOS_*
Burkitt lymphoma
_Burkitt-like lymphoma with 11q aberration_*
High-grade B-cell lymphoma, with _MYC_ and _BCL2_ and/or _BCL6_ rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma
Mature T and NK neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK-cell leukemia
Systemic EBV$^+$ T-cell lymphoma of childhood*
Hydroa vacciniforme–like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma*
_Indolent T-cell lymphoproliferative disorder of the GI tract_*
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma
_Primary cutaneous acral CD8$^+$ T-cell lymphoma_*
_Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder_*
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
_Follicular T-cell lymphoma_*
_Nodal peripheral T-cell lymphoma with TFH phenotype_*
Anaplastic large-cell lymphoma, ALK$^+$
Anaplastic large-cell lymphoma, ALK$^-$*
_Breast implant–associated anaplastic large-cell lymphoma_*
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

Provisional entities are listed in italics.
*Changes from the 2008 classification.

Table 17.C. Grading of selected central nervous system tumours (WHO 2016 classification)

Source: adapted from Louis DN, Ohgaki H, Wiestler OD *et al*. (2016) *World Health Organization histological classification of tumours of the central nervous system*. Geneva: WHO Press/Lyon: International Agency for Research on Cancer.

Diffuse astrocytic and oligodendroglial tumours	**I**	**II**	**III**	**IV**
Diffuse astrocytoma, IDH-mutant		•		
Anaplastic astrocytoma, IDH-mutant			•	
Glioblastoma, IDH-wildtype				•
Glioblastoma, IDH-mutant				•
Diffuse midline glioma, H3K27M-mutant				•
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted		•		
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted			•	

Other astrocytic tumours	**I**	**II**	**III**	**IV**
Pilocytic astrocytoma	•			
Subependymal giant cell astrocytoma	•			
Pieomorphicxanthoastrocytoma		•		
Anaplastic pleomorphic xanthoastro-cytoma			•	
Ependymal tumours	**I**	**II**	**III**	**IV**
Subependymoma	•			
Myxopapillaryependymoma	•			
Ependymoma		•		
Ependymoma, RELA fusion-positive		•	•	
Anaplastic ependymoma			•	
Other gliomas	**I**	**II**	**III**	**IV**
Angiocentricglioma	•			
Chordoidglioma of third ventricle		•		
Choroid plexus tumours	**I**	**II**	**III**	**IV**
Choroid plexus papilloma	•			
Atypical choroid plexus papilloma		•		

	I	II	III	IV
Choroid plexus carcinoma			•	
Tumours of the pineal region	**I**	**II**	**III**	**IV**
Pineocytoma	•			
Pineal parenchymal tumour of intermediate differentiation		•	•	
Pineoblastoma				•
Papillary tumour of the pineal region		•	•	
Meningiomas	**I**	**II**	**III**	**IV**
Meningioma	•			
Atypical meningioma		•		
Anaplastic (malignant) meningioma			•	

Embryonal tumours	**I**	**II**	**III**	**IV**
Medulloblastoma (all subtypes)				•
Embryonal tumour with multi-layered rosettes, C19MC-altered				•
Medulloepithelioma				•
CNS embryonal tumour, NOS				•
Atypical teratoid/rhabdoid tumour				•
CNS embryonal tumour with rhabdoid features				•

Neuronal and mixed neuronal-glial tumours	**I**	**II**	**III**	**IV**
Dysembryoplasticneuroepithelial tumour	•			
Gangliocytoma	•			
Ganglioglioma	•			
Anaplastic ganglioglioma			•	
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	•			
Desmoplastic infantile astrocytoma and ganglioglioma	•			
Papillary glioneuronal tumour	•			
Rosette-forming glioneuronal tumour	•			
Central neurocytoma		•		
Extraventricularneurocytoma		•		

Cerebellar liponeurocytoma		•		
Tumours of the cranial and paraspinal nerves	**I**	**II**	**III**	**IV**
Schwannoma	•			
Neurofibroma	•			
Perineurioma	•			
Malignant peripheral nerve sheath tumour (MPNST)		•	•	•
Mesenchymal, non-meningothelial tumours	**I**	**II**	**III**	**IV**
Solitary fibrous tumour/haemangiopericytoma	•	•	•	
Haemangioblastoma	•			
Tumours of the sellar region	**I**	**II**	**III**	**IV**
Craniopharyngioma	•			
Granular cell tumour	•			
Pituicytoma	•			
Spindle cell oncocytoma	•			

Table 17.D. Recommendations on the use of organs from donors with CNS tumours

Source: Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO). *Transplantation of organs from deceased donors with cancer or a history of cancer.* London: Department of Health and Social Care, April 2014 (Chapter 4, Ref. 21)

Absolute contraindications • Primary cerebral lymphoma • All secondary intracranial tumours
Intracranial tumours with an intermediate risk of cancer transmission (2.2 % with an upper 95 % CI of 6.4 %) include WHO grade 4 tumours and equivalents: • Glioblastoma • Giant cell glioblastoma • Gliosarcoma • Pineoblastoma • Medulloblastoma • CNS primitive neuroectodermal tumour • Medulloepithelioma • Ependymoblastoma • Atypical teratoid/rhabdoid tumour • Malignant peripheral nerve sheath tumour • Germinoma • Immature teratoma • Teratoma with malignant transformation • Yolk sac tumour • Embryonal carcinoma • Choriocarcinoma
Intracranial tumours with a low risk of transmission (<2 %) include WHO Grade 3 and equivalents: • Anaplastic astrocytoma • Anaplastic oligodendroglioma • Anaplastic oligoastrocytoma • Ependymoma • Choroid plexus carcinoma • Anaplastic gangliomyoma • Pineal parenchymal tumour of intermediate differentiation • Papillary tumour of the pineal region • Malignant peripheral sheath tumour • Anaplastic/malignant meningioma • Papillary meningioma • Rhabdoid meningioma • Haemangiopericytoma

Table 17.E. Recommendations on the use of organs from donors with non-CNS cancers

Source: Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO). *Transplantation of organs from deceased donors with cancer or a history of cancer*. London: Department of Health and Social Care, April 2014.

Absolute contraindications

- Active cancer with spread outside the organ
- Active haematological malignancy

High risk (>10 % risk of transmission)

- Melanoma: without spread (except as below)
- Breast: cancer other than those identified below
- Colon: cancer other than those identified below
- Kidney: renal cell cancer >7 cm or stages 2-6
- Sarcoma: >5 years previously and resected
- Small cell cancer: lung/neuroendocrine
- Lung cancer: stage I to IV

Low risk (0.1-2 % risk of transmission)

- Melanoma: superficial spreading type with tumour thickness <1.5 mm with curative surgery and cancer free period of >5 years
- Breast: stage 1, hormone receptor negative with curative surgery and cancer-free period of >5 years
- Ovary: curative surgery and cancer-free >10 years
- Colon: adenocarcinoma with curative surgery and cancer-free period of >5 years
- Thyroid: solitary papillary carcinoma 0.5-2.0 cm
- Thyroid: minimally invasive follicular carcinoma 1.0-2.0 cm
- Kidney: resected solitary renal cell carcinoma >1.0 cm and <2.5 cm and Fuhrman grade ½
- Prostate: Gleason >6
- Treated gastrointestinal stromal cancers

Minimal Risk (<0.1 % risk of transmission)

- Skin: basal cell carcinoma
- Skin: squamous cell carcinoma with no metastases
- Skin: non-melanoma skin cancer *in situ*
- Uterine cervix: *in situ* cancer
- Thyroid: solitary papillary carcinoma (<0.5 cm)
- Thyroid: minimally invasive follicular carcinoma (<1.0 cm)
- Bladder: superficial non-invasive papillary carcinoma
- Kidney: Resected solitary renal cell carcinoma <1.0cm and Fuhrman grade ½
- Prostate: Gleason <6 or >6 with curative treatment and cancer free >3 years

Note. Only those cancers where evidence is available for analysis have been classified.

Appendix 18. Sample haemodilution algorithm

DONOR ID # ____________________

Date and Time of Specimen Collection ______________________
Donor's weight in kg ____________________

A = Total volume of blood transfused in the 48 hours before death or sample collection, whichever comes first

B = Total volume of colloid infused in the 48 hours before death or sample collection, whichever comes first

C = Total volume of crystalloid infused in the 1 hour before death or sample collection, whichever comes first

BV = donor's blood volume

Calculated blood volume = donor's weight (kg) / 0.015 OR
donor's weight (kg) x 70 mL/kg

PV = donor's plasma volume

Calculated plasma volume = donor's weight (kg) / 0.025 OR
donor's weight (kg) x 40 mL/kg

Calculate both:

1. Is B + C > PV?
2. Is A + B + C > BV?

[Enter a zero if a category (A, B, or C) was not transfused/infused.]

Determination of Sample Acceptability for Infectious Disease Tests:

If the answers to both 1 and 2 are NO, the post-transfusion/infusion sample is acceptable.

If the answer to either 1 or 2 is YES, the post-transfusion/infusion sample is not acceptable; use a pre-transfusion/infusion sample or reject the donor

Source: US Food and Drug Administration. Guidance for industry: eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps), August 2007. Appendix 2, available at www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm091345.pdf, accessed 14 January 2019.

Appendix 19. Example of validation of screening- infectious disease assays for use with blood from deceased donors.

The reliability of the results of screening for infectious diseases in blood samples from deceased individuals is critical, and it can be enhanced substantially if appropriately validated assays are used. Assays for infectious diseases specifically labelled for use in screening blood donors are considered suitable for use in screening living donors of tissues and/or cells. However, few of these assays have been validated specifically for use with blood collected after the donor's heart has stopped beating. If an assay's manufacturer has not validated the assay for use with blood samples from deceased donors, no claims are made in regard to the performance or reliability of the test results generated with such samples. Consequently, screening laboratories are expected to specifically validate these assays with such blood samples to support performance of deceased-donor screening in their laboratory.

Before any validation work, the potential issues associated with screening blood from deceased individuals must be understood. There are three key issues:

a. occurrence of *post mortem* degradation, or fall in detectable level, of a screening target (a marker of infectious disease, such as an antigen or antibody related to the infectious agent);

b. inhibition of the assay by substances accumulating from *post mortem* changes in blood;

c. the potential for a blood sample from a deceased donor to be haemodiluted.

In all three cases there is the possibility of a false-negative test result. Although sensitivity and specificity are important for any screening assay, whether the samples being tested are from living or deceased individuals, sensitivity is the more important of the two because a false-negative result is the major threat in regard to the safety of the tissue or cell transplant. In general, specificity is not as important because algorithms can be employed to effectively discriminate between non-specific and specific reactivities in screening assays.

To validate assay performance when using blood samples from deceased donors, the following recommendations apply.

a. The collection times for blood samples from deceased donors used for assay validation must be representative of the full range of time points typically encountered during tissue procurement, specifically from immediately after death up to 24 h after death (see §5.3.1.1).

b. All information about storage and handling conditions for blood tubes from time of blood collection to time of testing must be documented and meet any assay sample-handling requirements stated.

c. Each blood sample from a deceased donor used for validation must be evaluated for haemodilution using an approved algorithm.

d. Use a dilution series prepared in deceased-donor material; or use spiked specimens inoculated with the relevant infectious-disease marker at a potency near the assay's cut-off, and vary the sources used for spiking. In both cases, test in parallel with the same material diluted in serum or plasma from a living individual.

e. Test a sufficient number of samples from different deceased donors (≥20).

f. Include haemolysed samples.

g. The sample storage methods (i.e. refrigerated, frozen) used for validation should mimic the method of storage that is routine for that laboratory.

Assay evaluation is undertaken to determine the overall performance of an assay, specifically including its core sensitivity and specificity.

Validation is done to determine the suitability of an already evaluated assay for use for a specific purpose in a laboratory; for example, for use with a blood specimen type (i.e. from a deceased donor) not specifically stated as known to be acceptable by the manufacturer. Evaluations are undertaken using panels of provenanced samples of known status, and importantly include seroconversion panels (i.e. sets of sequential samples from

infected individuals following the course of infection from pre-exposure to late infection). However, such samples are almost all obtained from living patients. Suitable comparable ‘positive’ specimens in a series from deceased individuals are not available, making this specific type of evaluation not possible. An alternative, less realistic approach but one that has been accepted by licensing authorities is to spike non-reactive blood specimens from deceased donors with known and varying levels of virus to attempt to mimic a ‘true’ positive specimen [1, 2, 3].

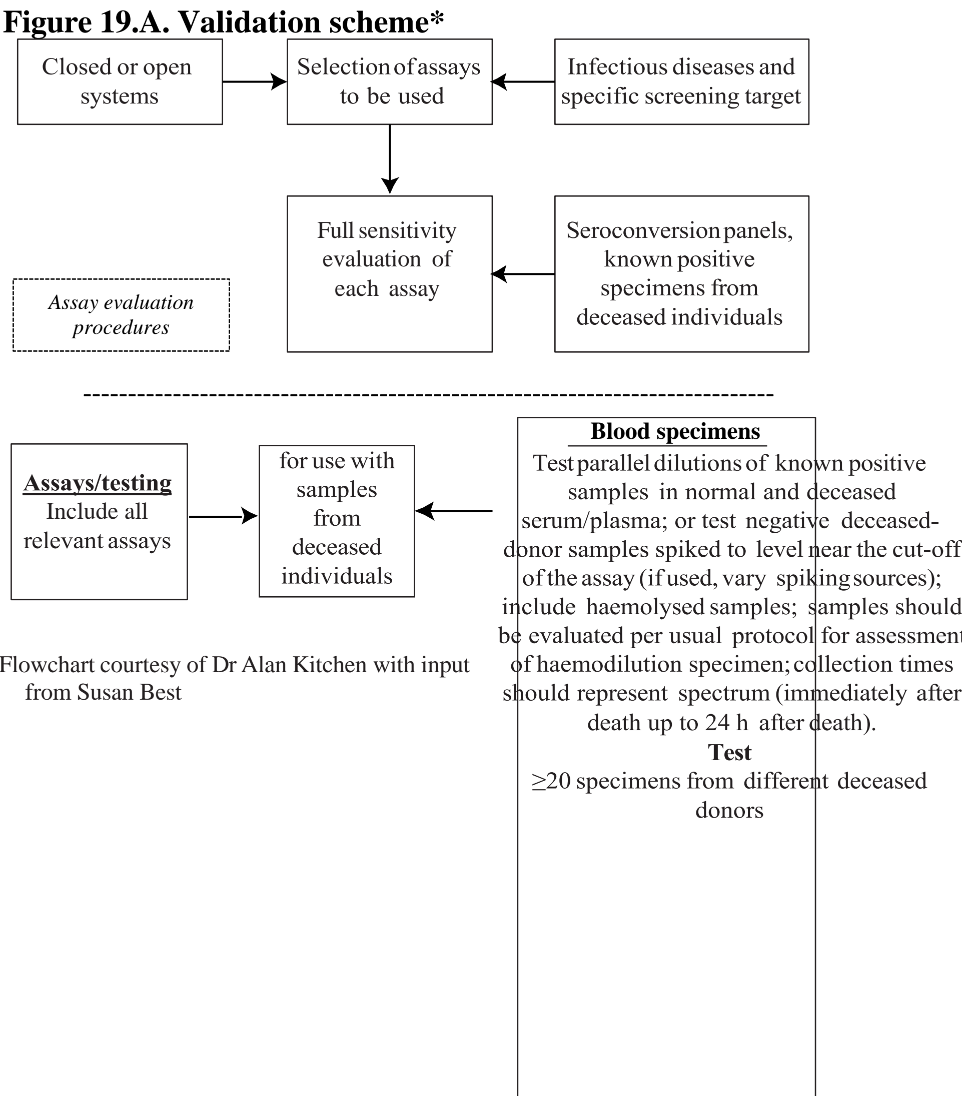
Nonetheless, before using assays with blood specimens from deceased individuals, the performance of the assays must be assessed in some way to make attempts to demonstrate that there is no loss of, or other change in, the expected performance of the assay.

Validation of assays for use with blood from deceased individuals is critical, both for serological assays that detect antigens or antibodies and for molecular assays that detect viral nucleic acid. However, simultaneously, this is problematic because of the key issues stated above and a lack of suitable blood samples from deceased individuals to support such work. The issue of the degradation of any screening target that may be present is the hardest to deal with, because of the lack of suitable published studies. However, if the timeframe for sample collection *post mortem* is limited by those carrying out tissue procurement, it can be theorised that it would be unlikely that any markers of a previously unidentified and relevant infection would have been degraded to a level that is undetectable using the high-quality assays available from major international diagnostics manufacturers.

If this aspect is controlled, the next issue to be considered is the potential ‘inhibitory effect’ of the sample as a result of any *post mortem* changes. To some degree the same argument applies in regard to degradation of the screening target *post mortem* because specimen collection within a suitable timeframe minimises the extent of any *post mortem* changes, whatever they are (e.g. red cell haemolysis, precipitants/byproducts of cell death). There are ways in which an inhibitory effect can be examined in serological and molecular assays. Known positive serum/plasma samples can be diluted in serum plasma from living individuals in parallel with serum/plasma from deceased individuals and from living individuals, either to a fixed point or to extinction, and the outcomes compared. In this aspect, validation of molecular assays is slightly easier than that of serological assays because the inclusion of an internal control in molecular assays validates each test result with respect to the presence of any ‘inhibitory substance’ that may be present in a sample. If the internal control is not amplified, the test result for the sample is deemed to be invalid. Therefore, although not definitive, such approaches would identify any gross effects, which would most likely result in false-negative results.

To meet donor/donation screening expectations, validation of infectious-disease assays for use with blood from deceased individuals must be undertaken. If the assays have been evaluated appropriately by the testing laboratory, then there is clear understanding of the baseline performance of each assay, and this can be used as the basis of additional assay validation work for use with blood samples from deceased donors. Lack of suitable samples to mirror evaluation panels is a particular problem, leaving comparative dilutional studies and/or spiked sample studies as feasible approaches to determine any problems associated with testing blood from deceased individuals.

Figure 19.A. Validation scheme*



*Flowchart courtesy of Dr Alan Kitchen with input from Susan Best

References

1. Edler C, Wulff B, Schroeder AS *et al*. A prospective time course study on serological testing for human immunodeficiency virus, hepatitis B virus and hepatitis C virus with blood samples taken up to 48 hours after death. *J Med Microbiol* 2011;60:920-6.

2. Meyer T, Polywka S, Wulff B *et al*. Virus NAT for HIV, HBV, and HCV in post-mortal blood specimens over 48 h after death of infected patients – first results. *Transfus Med Hemother* 2012 Dec;**39**(6):376-80.

3. Commission Directive 2012/39/EU of 26 November 2012 amending Directive 2006/17/EC with regard to certain technical requirements for the testing of human tissues and cells, available at eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:327:0024:0025:en:PDF, accessed 13 January 2019.

Appendix 20. *Treponema pallidum* testing

The algorithms that are recommended for the serological diagnosis of syphilis are challenging because of the inherent complexity of these methods. These tests are subdivided into treponemal and non-treponemal tests, and the interpretation of the results obtained is often particularly difficult, hence the need for further confirmatory testing (see Table 20.A). See also the website of the European Centre for Disease Prevention and Control at www.ecdc.europa.eu/en/Pages/home.aspx.

Non-treponemal tests

Non-treponemal tests are tests that search for IgG and IgM directed against the lipids that are released from the damaged human cells during an early stage of the disease. The goal of these tests is therefore to search for antibodies to antigens that are not specific to an infection with species of the genus *Treponema*, as reflected in the term reaginic antibodies. The non-specific nature of this category of serological tests is also reflected in the fact that many other causes, such as advanced age, pregnancy, various types of malignant tumours, autoimmune diseases and other unrelated infections may result in the formation of anti-lipoid antibodies, thus generating false-positive results.

Consequently, a positive result obtained with a non-treponemal test should always be confirmed by means of a treponemal test. Moreover, non-treponemal tests usually display a low sensitivity in the detection of early syphilis, partly because the first positive results are not obtained until 4-8 weeks after infection. The tests belonging to this category have mainly a diagnostic purpose as part of the therapeutic follow-up of patients with syphilis. Thus, a declining titre over a certain period of time is indicative of a favourable response to treatment. As a rule, successful treatment leads to negative results for these tests. The Venereal Diseases Research Laboratory (VDRL) test and the Rapid Plasma Reagin (RPR) test belong to this group of non-treponemal tests used for serological syphilis screening.

Treponemal tests

Treponemal tests are serological screening tests that search for specific antibodies directed against species of the genus *Treponema*. No distinction can be made between the different treponematoses due to immunological cross-reactions. These tests usually remain positive after the initial infection, which means that they cannot be used to monitor the response to treatment or diagnose reinfections. Treponemal serological tests include the *T. pallidum* haemagglutination (TPHA) test, the *T. pallidum* particle agglutination (TPPA) test, treponemal enzyme immunoassays (EIA), chemiluminescence immunoassays (CLIA) and immunoblotting.

Algorithms in use

New developments, especially in the optimisation of treponemal immunoassays, offer new possibilities due to the earlier detection of syphilis and the shorter diagnostic window, but do not necessarily simplify the assessment of the overall serological picture. According to recent international recommendations, the following screening algorithms can be used for serological syphilis screening.

Only the treponemal screening test

This screening strategy is commonly used in European blood banks and laboratories because of its potential for large-scale automation. This algorithm identifies both those individuals in whom syphilis has been treated successfully as well as those who have not received any treatment. It is better suited to detecting the early stages of infection than the sole use of a non-treponemal test. Given the fact that this strategy is mainly used for populations with a low prevalence of syphilis, it suffers from a considerable number of false-positive results.

Only the non-treponemal screening test

Ideally, a non-treponemal test carried out for screening purposes should be quantitative in nature in order to rule out the prozone effect when using undiluted blood samples. (This concerns <2 % of samples, usually during the secondary phase of syphilis. These patients display extremely high titres of antibodies that interfere with the formation of antigen–antibody complexes, which are necessary to visualise flocculation when interpreting the non-treponemal test.) This algorithm can only detect active (infectious) syphilis, which means that it can miss the early stage of syphilis.

Treponemal and non-treponemal tests

This algorithm is especially useful to screen high-risk populations as well as to screen for the early stages of syphilis.

In the serological diagnosis of syphilis and independently of the screening algorithm used, a confirmatory test will always need to be carried out, regardless of which of the screening tests turned out positive:

- If the initial screening test included only a treponemal test, the results should be confirmed by means of a second treponemal test based on a different analytical method, as well as a quantitative non-treponemal test if this second treponemal test also turns out positive.
- If the initial screening test included only a non-treponemal test, the positive result needs to be confirmed by means of a treponemal test, whereas the non-treponemal test should be performed in a quantitative manner if this was not initially the case.
- If the initial screening was performed using a treponemal test as well as a non-treponemal test, the non-treponemal test should be performed in a quantitative manner. A second treponemal test based on a different analytical method may be used to rule out a false-positive result for the initial treponemal test only if the non-treponemal test is negative.

Table 20.A. Interpretation of the results of biological screening tests for syphilis

Treponemal test	*Non-treponemal test*	*Interpretation*	*Consequence for the donation*	*Further management*
positive	positive[1]	active infection	reject	contact the attending physician
positive	negative	treated (past) infection *or* early stage of infection *or* false-positive[2]	reject	contact the attending physician[2]
negative	not carried out *or* negative	no infection	release possible	no further action
negative	positive	false-positive result for the non-treponemal test *or* false-negative result for the treponemal test	release potentially possible[3]	no further action

Notes

[1] Given the fact that in the vast majority of cases in which the non-treponemal test yielded false-positive results, the titres were ≤ 1/4, a "positive treponemal test" is considered to be with a titre ≥ 1/8.

[2] In such a case, a confirmatory treponemal test needs to be carried out. If this confirmatory treponemal test yields a negative result, the initially positive result of the treponemal test is not confirmed and therefore looked upon as false-positive, which justifies release of tissues and cells intended for donation and requires no contact with the attending physician concerned.

[3] The tissue establishment administrator can still accept the tissues and cells after having consulted the clinical biologist, possibly after carrying out additional tests, and having received the informed consent of the recipient and the medical transplant team.

(extracted from NHS FRM3831/5.1)

Tissue Services Tissue Donation From Deceased Donors

Venue:	Donation Number and Donor Number:

The intent of the below is to assess the suitability of the working environment for safety to both staff and tissue.

Minimum Requirements:

	Yes	No	Comments
Are there a minimum 2 authorised people present?			
Is there good lighting and a sink with running water available?			
Do you know the evacuation procedure?			
Is there suitable access to minimise carrying and handling e.g. parking.			
Is the support equipment working and used where applicable? e.g. trolleys, control panels, 'in use' signs etc.			
Can unauthorised people view the donation? e.g. doors open, blinds open, clear glassed windows			
Is there unauthorised filming / photography equipment in use?			

Site Assessment:

	Yes	No	Comments / Action
Are the floors wet?			
Do any surfaces appear dirty / contaminated?			
Are there any sharp objects/dangerous equipment/clutter around i.e. hazards to avoid/move?			
Is the donation area a clean environment (if necessary clean with detergent prior to use)?			
Is a post mortem being carried out at the same time as the donation?			
Do you believe that tissue can be retrieved with minimal or no environmental contamination?			
Are there specific donor related risks and actions taken to mitigate these risks e.g. large donor?			

	Yes	No	Comments / Action
Was the Donors face protected prior to moving to donation area?			If No, explain.
Person Responsible for moving donor to / from donation area:	Delete as applicable: **Porters / APTs / TS Staff / Others** Others please specify:		

Authorisation to proceed:

Are you satisfied that this is a safe / clean working environment? Yes / No If No, contact the Duty Manager ASAP Name of Manager contacted:

Signed: **Date:**

(extracted from NHS FORM FRM3831/5.1) Effective: 16/12/13

Tissue Services Tissue Donation From Deceased Donors

Donation Number:		
Confirm correct donor by transcribing identification details directly from identity band/label. You **must** have 3 points of matched ID (e.g. name, dob, hospital number, address, or (exceptionally) circumstances of death). Method of ID: Toe tag/Identity band/Mortuary label/Other………………………………. **COPY TAG**		
Identification and Examination performed by (PRINT NAME)	Sign:	Date:
Identification Double checked by (PRINT NAME)	Sign:	Date:

Check Authorisation. Do you have 3 points of matched ID that correspond with the referral information and donor identification?			
Yes / No	Print name:	Sign:	Date:
If No, can you justify your rationale for proceeding with the donation below? Yes / No / NA **If No, contact Duty Manager ASAP** Name of Manager contacted:			

ADDITIONAL INFORMATION	SIGNATURE AND DATE

Appendix 23. Checklist for revision of computerised systems

Establishment:

Date

Signature of person responsible for the revision:

1. Identification of the system and function	Comments
Name of the system: Version: Supplier: Platform: Function: Connected with other computerised systems:	
2. Organization	
☐ Is the organisation for function and maintenance described in the QM system? (system owner, system manager, person responsible) ☐ Are these functions placed in an organisation scheme?	
☐ Is the responsibility of the supplier described in QM system and in written agreements? ☐ Is it clear that the user is responsible for validation when data are transferred between different systems?	
☐ Have the responsible persons (functions) received enough and documented training in case of malfunction of the system?	
☐ Is the computerised system included in the scheme for internal revision?	
3. Written agreements	
☐ Are responsibilities for support (software and hardware) clearly defined? ☐ Are functions/responsibilities of subcontractors included?	

☐ Are instructions for documentation of unexpected events included? ☐ Is time limit for corrective actions by responsible support defined?	
If data are transferred between different computerised systems:	
☐ Are platforms and protocols described? ☐ Are obligations to inform each other, about changes and events that may influence information transfer, included? ☐ Are responsibilities for the different parts in the chain between the systems clearly defined?	
4. Documentation of the system	
☐ Is complete and updated documentation of the system accessible? ☐ Does the documentation contain measures for managing malfunctions and fallbacks? ☐ Is a user guide with version number accessible? - in paper copy - as electronic "help-function"	
5. Maintenance	
☐ Are standard operating procedures available for measures in case of malfunction/ total downtime? ☐ Back-up system? ☐ Reset of data? ☐ Are back-up system and read-back functions tested?	
6. Changes	
☐ Is a test environment available? ☐ Are validation procedures defined and performed before updates, changes, new versions in the system?	
7. Information security	
☐ Is access to the computers protected by locked doors? (physical data protection)	

☐ Is a virus protection system active? (if applicable)	
☐ Is access to the computerised system protected by personal login? ☐ Single-level login ☐ Double-level login	
☐ Is access to the system (and login) associated with a certificate of authorisation? ☐ Who decides on, and keep records of, access to the system? ☐ Are records for access to the system updated (i.e. access removed when not needed anymore)?	
☐ Does the system provide traceability of the user? ☐ Does the system provide traceability of changes in manually added data/ text? (with the original text still readable)	
If data are manually inserted/ transferred from another system: How is correctness of the data verified? ☐ By data insert of two individuals independently ☐ By saving the original (paper) result ☐ By signature(s) of the individual(s) inserting the data	
If data are automatically transferred from another computerised system: ☐ Are "check points" to verify the correctness of data transfer available in the system or as standard operating procedures?	

Adapted from SWEDAC DOC 10:5, 2010 Guidance for information security managers (available from www.isaca.org/Knowledge-Center/Research/ResearchDeliverables/Pages/Information-Security-Governance-Guidance-for-Information-Security-Managers.aspx) with interpretation of ISO/IEC 17025 "General requirements for the competence of testing and calibration laboratories" (available from www.iso.org/iso/catalogue_detail?csnumber=39883) and ISO/IEC 27007:2011 (available from www.iso.org/obp/ui/#iso:std:iso-iec:27007:ed-1:v1:en).

Appendix 24. Serious Adverse Reaction or Event impact assessment form

This impact assessment tool assists practitioners and regulators in planning their response to a given serious adverse reaction or event (SARE), taking into account the broader consequences beyond the individual patient affected or potentially affected. The assessment should be based on available data, past experience and scientific expertise.

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	**Rare**	Difficult to believe it could happen again
2	**Unlikely**	Not expected to happen again
3	**Possible**	May occur occasionally
4	**Likely**	Expected to happen again, but not persistently
5	**Probable**	Expected to happen again on many occasions

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		**On individual(s)**		**On the system**		**On tissue/cell supply**
0	**Insignificant**	Nil	**OR**	No effect	**OR**	Insignificant
1	**Minor**	Non-serious	**OR**	Minor damage	**OR**	Some applications postponed
2	**Moderate**	Serious	**OR**	Damage for short period	**OR**	Many cancellations or postponements
3	**Major**	Life-threatening	**OR**	Major damage to system – significant delay to repair	**OR**	Significant cancellations – importation required
4	**Catastrophic or extreme**	Death	**OR**	System destroyed – need to rebuild	**OR**	All allogeneic applications cancelled

Step 3: Application of the impact matrix

Likelihood of recurrence ⇨ / ⇩ **Impact of recurrence**	**1 Rare**	**2 Unlikely**	**3 Possible**	**4 Likely**	**5 Certain /almost certain**
0 Insignificant	**0**	**0**	**0**	**0**	**0**
1 Minor	**1**	**2**	**3**	**4**	**5**
2 Moderate	**2**	**4**	**6**	**8**	**10**
3 Major	**3**	**6**	**9**	**12**	**15**
4 Catastrophic/extreme	**4**	**8**	**12**	**16**	**20**

Step 4:

The response of a tissue establishment or Health Authority to a specific SARE should be proportionate to the potential impact, as assessed by the matrix shown in Step 3 above and described below.

White: The tissue establishment is to manage the corrective and preventive actions; the Health Authority is to file the report and keep a 'watching brief'.

Pale grey: Requires interaction between the tissue establishment and the Health Authority, which may request an inspection that focuses on the SARE and the corrective and preventive actions to be followed up, including evidence of effective recall, where necessary. Written communication to professionals working in the field might be appropriate.

Dark grey: The Health Authority will, in general, designate representatives to participate in developing or approving the corrective and preventive action plan (possibly a task force to address broader implications). Inspection, follow-up and written communication should be done as at the previous level; and possibly notification of Health Authorities in other countries where relevant.

Effectiveness of the response can be assessed by re-applying the impact matrix following implementation of corrective and preventive actions. The impact can be reduced by:

a. reducing the probability of recurrence through preventive measures;
b. increasing the detectability of the risk; or
c. reducing the severity of the consequences, if it should recur.

Appendix 25. Serious Adverse Reaction notification form for ocular tissues, (Agence de la Biomédecine, France)

Notification Form for SAR on Ocular Tissues							
SEC		**Récipient code**		**Birth date**			
Date of transplant				**Sexe**	❑ Male	❑ Female	
				Eye involved	❑ Right	❑Left	

Type de greffe : ❑ DALK ❑ DSAEK ❑ DMEK ❑ PK

SAR
1- Short term (per-graft until 1 month post-graft)
❑ Primary graft failure (endothelial decompensation)
❑ Ocular infection (from bacterial, fungal, parasitic or viral origin including endophthalmitis)
❑ Irreversible rejection (specific immunologic response)
❑ Systemic infection (compatible with a donor-recipient transmission).
❑ Persistent ulceration or corneal perforation

2- Mid to long term	**1 month to 1 year**	**> 1 year**
❑ Any ocular pathology that could suggest transmission from the donor (for example: tumoral pathologies of the anterior segment)	☐	☐
❑ Any systemic pathology that could suggest transmission from the donor (transmissible viral diseases (rabies, hepatitis, etc.), malignant diseases, prion disease ...)	☐	☐
❑ Unrecognized donor corneal diseases (history of refractive surgery, corneal dystrophy including FUCHS, keratoconus, scars ....)	☐	☐
❑ Endothelial cell decompensation including cornea guttata	☐	☐
❑ Chronic endophthalmitis	☐	☐
❑ Late-onset local infection including bacterial, fungal, viral, or parasitic keratitis or kerato-endotheliitis.	☐	☐
❑ Failure (leading to re-graft) precise : :………………………………	☐	*
❑ Persistence of complicated epithelial defects (epithelial ulcerations) (visual decline, infection, rejection, stromal ulceration, perforation ...) or development of epithelial tumor, dysplasia or epithelial hyperplasia	☐	☐
❑ Defect of corneal transparency (corneal opacification, calcifications, corneal transparency delay, inflammatory infiltrate ...)	☐	☐
❑ Astigmatism induced by the graft or giant astigmatism (> 8 D) linked or likely to be linked to recurrence or appearance of keratoconus on the graft	☐	☐
❑ Loss of Eyeball (anatomical or functional)	☐	☐
❑ Death of patient linked to the ocular graft	☐	☐

Risk Factors

❑ No risk factor identified

❑ Risk factor identified (tick the one concerned)

- Neovascularization - History of autoimmune disease (scarring pemphigoid)
- graft size outside the range of 8.5diameter
- Hypertonia / glaucoma
- Ocular inflammation / uveitis
- Neurotrophic history
- Palpebral alterations (statics / kinetics)
- Emergency cornea grafting or therapeutic keratoplasty
- history of rejection on either ipsi or contralateral eye
- Atopic ground
- Pediatric patient or less than 16 years old
- Re-graft (how many: ....)
- Herpesvirus infection (HSV, VZV)
- Dry eye syndrome
- Chemical burn
- Other, gives details ………

FORM FRM4159/2 Effective: 17/07/17

Ocular Tissue Transplantation - Serious Adverse Reactions/Events

Part A – NOTIFICATION OF ADVERSE REACTIONS AND EVENTS

(See reverse side of this page for important additional information)

Please complete this Part A form to notify NHSBT of an Adverse Reaction/Event:

- Contact Tissue and Eye Services Customer Care on Tel: 08456076819
- Send one copy of this form to Tissue and Eye Services Customer Care either by e-mail to tscustserv@nhsbt.nhs.uk, or by fax to 08456076820, or by post to Tissue and Eye Services Customer Care Dept., NHSBT, 14 Estuary Banks, Speke, Liverpool L248RB; you can also report online at https://www.organdonation.nhs.uk/IncidentSubmission
- Retain original in the patient's notes

DONOR NUMBER/ EYE BANK REFERENCE ....

REPORTING DATE .... **RECIPIENT INITIALS & DATE of BIRTH** ....

DATE OF TRANSPLANT .... **RECIPIENT NHS/CHI NUMBER** ....

CONSULTANT (please print) .... **RECIPIENT HOSPITAL** ....

CONTACT DETAILS Tel .... **Email** ....

INDICATION / URGENCY of TRANSPLANT..

EYE BANK SUPPLYING THE TISSUE please tick)

□ Filton/Bristol □ Manchester □ Moorfields □ East Grinstead □ other

TYPE OF TISSUE OR CELLS (please tick)

□ Cornea □ Sclera □ Limbal

□ Other - please specify...

ADVERSE REACTION

DATE OF SUSPECTED ADVERSE REACTION ....

TYPE OF SERIOUS ADVERSE REACTION (please tick)

1. Primary graft failure (corneal transplant never cleared)
2. Endophthalmitis or other serious ophthalmic infection
3. Graft failure due to donor tissue which was out of date, scarred or had evidence of previous surgery.
4. Malignancy likely to be attributable to the transplanted tissue
5. Systemic infection possibly attributable to the transplanted tissue
6. Other, please specify ....

ADVERSE EVENT

TYPE OF SERIOUS ADVERSE EVENT (please tick)

1. Wrong material supplied.
2. Tissue supplied is out of date.
3. Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery.
4. Other, please specify ....

Was there any adverse impact on the patient, e.g. patient already anaesthetized?...

WAS THIS TISSUE USED? YES / NO

PLEASE RETURN ALL UNUSED TISSUE IN THE ORIGINAL CONTAINER TO THE ISSUING EYE BANK FOR INVESTIGATION; PLEASE DO NOT USE FORMALIN

(Template Version 01/11/13)

Ocular Tissue Transplantation - Serious Adverse Reactions/Events

Ocular Tissue Transplantation - Adverse Reactions/Events

NHSBT SERIOUS ADVERSE REACTIONS/EVENTS REPORTING MECHANISM
Part A (i.e., this form) – To be completed by the person notifying NHSBT of an Adverse Reaction/Event
Serious Adverse Reactions/Events will be reviewed by NHSBT and OTAG[1]

DEFINITIONS[2]
'Serious adverse reaction'– means an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity

'Serious adverse event'– means any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalisation or morbidity.

Serious Adverse Reactions
Corneal Transplantation
1. Primary graft failure (graft never cleared)
2. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue[3]
3. Graft failure due to donor tissue which was out of date, damaged, scarred or had evidence of previous surgery
4. Malignancy likely to be attributable to the transplanted tissue
5. Systemic infection likely to be attributable to the transplant tissue
6. Other[4]

Ocular Tissue Stem Cell Graft
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue/cells[3]
2. Graft failure due to donor tissue/cells that were out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue/cells
4. Systemic infection likely to be attributable to the transplanted tissue/cells
5. Other[4]

Scleral Grafts
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue
2. Graft failure due to donor tissue which was out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue
4. Systemic infection likely to be attributable to the transplanted tissue
5. Other

Serious Adverse Events
There are potentially numerous serious adverse events. Specific serious adverse events that are peculiar to ophthalmology include
1. Wrong material supplied
2. Tissue supplied is out of date
3. Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery
4. Other[5]

Notes:
1. OTAG Adverse Reactions and Events Reporting subgroup
2. EU Tissues and Cells Directive 2004/23/EC
3. This relates to the development of a severe infection likely to be attributable to the transplanted tissue. It does not include for example the occurrence of a microbial keratitis related to a suture abscess.
4. Other refers to any unexpected adverse reaction which is considered by the surgeon to be serious and possibly attributable to the transplanted tissue
5. Other refers to any adverse event which is considered to be serious and could potentially lead to a serious adverse reaction if the tissue is used in a patient.

Appendix 27. Sample forms for evaluation of heart valves

Pulmonary Heart Valve Information

GRAFT INFORMATION	
Heart Valve Bank:	Donor Number:
ODT Number(if applicable):	Valve Number:
Date Dissected:	Date Cryopreserved:
Expiry Date of Valve:	Photograph Available on request: Y / N

L R R L

Pathology noted on cusps:

Key (please annotate the diagram above)
Atheroma = ………
Fenestration = 000
Fibrosis = XXXX
Other:

Condition: Excellent / Good / Fair

Comments:

Pulmonary Inner Annular Diameter	mm	Left artery inner annular diameter	mm
Length of Vessel	mm	Left artery length	mm
Length of muscle skirt (Min / max)	mm	Right artery inner annular diameter	mm
Total Length	mm	Right artery length	mm

STERILITY REPORT	
Hepatitis B:	HCV PCR:
Hepatitis B Core Antibody:	HIV PCR:
Hepatitis C:	HBC PCR :
HIV I and II:	Microbiology Culture:
Syphilis:	Mycobacteria:
HTLV :	Other :

DONOR INFORMATION	
Age:	Sex:
Date of Death:	Cause of Death:

Information Entered By:	**Date:**	**Signature:**

Aortic Heart Valve Information

GRAFT INFORMATION	
Heart Valve Bank:	Donor Number:
ODT Number (if applicable):	Valve Number:
Date Dissected:	Date Cryopreserved:
Expiry Date of Valve:	Photograph available on request: Y / N

R L L R

Pathology noted on cusps:

NCC
LCC RCC

Key (annotate diagram above)	
Atheroma = ………	LCC = Left Coronary Cusp
Fenestration = 000	RCC = Right Coronary Cusp
Fibrosis = XXXX	NCC = Non Coronary Cusp
Other:	

Aorta Inner Annular Diameter	mm
Length of Aorta	mm
Length of muscle skirt (Min / max)	mm
Total Length	mm

Condition: Excellent / Good / Fair

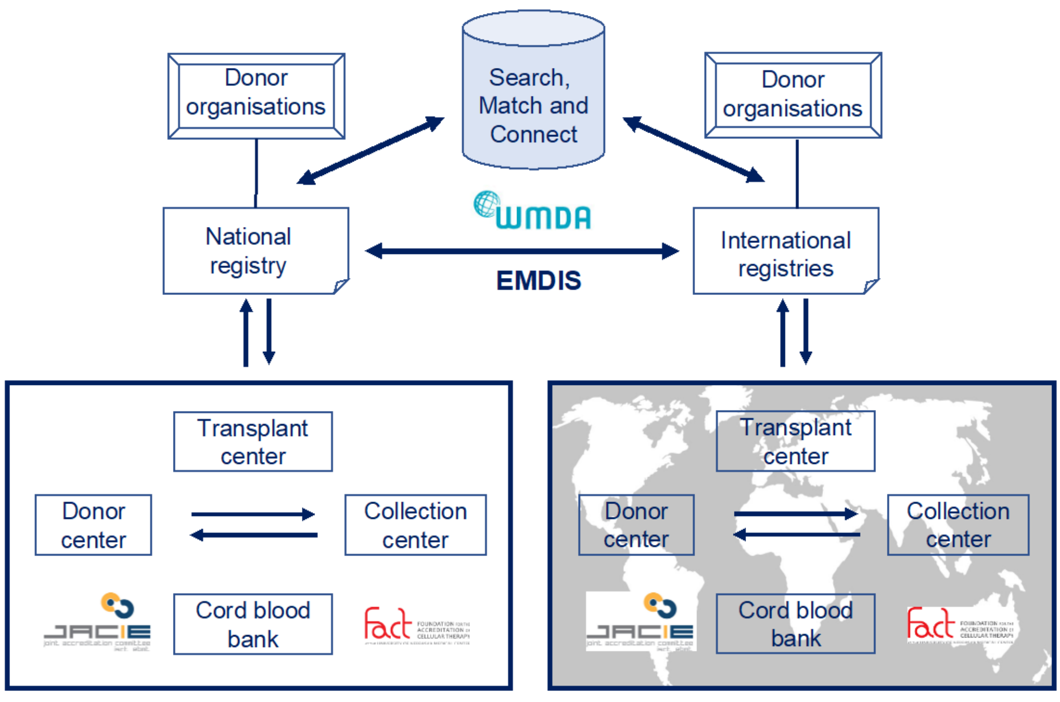
Comments:

SEROLOGY / BACTERIOLOGY STATUS		
Hepatitis B:	Hepatitis B Core Antibody:	Hepatitis C:
HIV I and II:	HIV PCR:	HCV PCR:
HBC PCR:	Syphilis:	HTLV:
Mycobacteria:	Microbiology Culture:	
ABO Blood group (if known):	Other:	

DONOR INFORMATION	
Age	Sex:
Date of Death:	Cause of Death

Information Entered By:	**Date**	**Signature**

Appendix 28. Donor search through registries for haematopoietic progenitor cells transplantation



WMDA: World Marrow Donation Association. **EMDIS**: European Marrow Donor Information System. **JACIE**: Joint Accreditation Committee ISCT-Europe & European Society for Blood and Marrow Transplantation. **FACT**: Foundation for the Accreditation of Cellular Therapy

Appendix 29. What to report and what not to report

The World Marrow Donor Association collects reports of adverse incidents in its Serious Event and Adverse Reaction (SEAR) and Serious Product Event and Adverse Reaction (SPEAR) programmes. The reports follow its standard operating procedure (SOP), which states its principles and gives examples, as listed below.

If an event/reaction is deemed to be one of the following, it should be reported if it meets these requirements:

a. Serious/unexpected/medically relevant/previously unknown.
b. Hospitalisation per se should NOT be reported, unless for an event that is life-threatening or fatal or unexpected.
c. Expected events (e.g. nausea/pain) should NOT be reported unless life-threatening or fatal.
d. Cell counts less than requested or expected should not be reported as a S(P)EAR unless a clinical consequence occurred or an error was responsible for the low count.

In addition to these general principles, the following specific events are given as indicators what should be reported:

SEAR SPECIFIC EXAMPLES

- Any serious event or reaction during anaesthesia should be reported.
  - E.g. profound bradycardia during anaesthesia requiring emergency treatment, laryngospasm during anaesthesia, severe adverse reactions to drugs or IV fluids
- Any serious cardiac complication should be reported.
- Any serious infection should be reported.
  - E.g. infections at site of marrow collection/line infections, sepsis, osteomyelitis
- Any serious mechanical injury should be reported.
  - E.g. nerve damage from marrow collection or IV lines, damage to SI joint, fractures of iliac crest, retroperitoneal haematoma or injuries
- Any serious incident in haemostasis should be reported
  - E.g. thrombosis, embolism, after marrow or PBSC harvest, abnormal bleeding secondary to thrombopaenia complicating PBSC harvest
- Any serious (late) effect of marrow or PBSC donation should be reported.
  - E.g. auto-immune, malignancy
- Any donor death (from initiation of donation until day 30 post-donation; or at any time if the donation is implicated).

SPEAR SPECIFIC EXAMPLES

Processing, labelling, handling and transport errors/problems

- Wrong stem cell product transfused
- Wrong stem cell product received
- Serious problems in transportation
- Damage to bag
- Inadequate cell dose in the stem cell product
- Clotting or other loss of product viability
- Contamination leading to serious infection in recipient

Any serious unpredicted transmissible infection

- HIV, Hepatitis B, Hepatitis C
- Not to be reported: CMV-positivity, EBV-positivity
- Not to be reported: contamination in product without infection in recipient

Any serious unpredicted non-infectious transmissible disease (e.g. malignant)

- E.g. malignancy, auto-immune disease, congenital anomaly

Source: adapted from WMDA (World Marrow Donor Association)

Appendix 30. Health assessment questionnaire cord blood donors

Mother name:
Collection facility: ISBT code:
Birth date: Questionnaire date:

POST BIRTH QUESTIONNAIRE	YES	NO	Observations
Pharmacological treatments history			
Did you take any medication in the last seven days before delivery?			
Did you take finasteride or similar medication for hair loss during pregnancy?			
Did you take Etretinate or similar medication for skin problems during pregnancy?			
In the last two weeks before birth			
Did you have fever with headache and malaise?			
Did you visit the dentist?			
In the last month before birth			
Did you get any vaccine?			
Were you in contact with someone who suffers a contagious infectious disease?			
In the last four months before birth			
Have you been to a doctor or been hospitalized?			
Did you get any endoscopy: colonoscopy, gastroscopy, cystoscopy or other?			
Did you get acupuncture with no disposable material?			
Did you get tattoo and / or piercing?			
Did you have contact with blood of another person by an accidental needle stick or splash?			
Have you lived or maintained close contact with a person with hepatitis, jaundice or hepatitis virus carrier?			
Did you get surgery?			
In the last six months before delivery			
Have you travelled outside Spain?			
Where?			
Did you ever live abroad (outside Spain)?			
Where?			
Have you travelled abroad?			
Where?			
Have you lived for more than one year -adding all periods of stay- in the United Kingdom (England, Wales, Scotland, Northern Ireland, Channel Islands, Isle of Man) during 1980-1996 period?*			
During pregnancy, have you resided in or travelled to a risk area for the Zika virus?			
During pregnancy, have you had a medical diagnosis of Zika virus infection?			
During pregnancy, have you had sexual contact with a male who: - Was diagnosed with a Zika virus infection in the 6 months prior to the sexual contact? - Travelled to or resided in a risk area for the Zika virus in the 6 months prior to the sexual contact?			
Have you ever been excluded as a blood donor?			

Have you had any serious disease requiring regular medical checking?			
Did you have a hepatitis, jaundice or liver problems?			
Have you been affected by any infectious disease such as malaria, Chagas disease, leishmaniasis, infectious tuberculosis, syphilis or gonorrhea, mononucleosis, among others?			
Did you have any serious illness of lungs, brain, kidneys, thyroid, digestive system or in other locations?			
Have you had heart or blood pressure problems?			
Are you diabetic treated with insulin, excluding gestational diabetes?			
Have you had any type of cancer?			
Have you had any illness or severe allergic reaction?			
Have you had any bleeding problem or blood disease, such as anaemia or excessive red blood cells?			
Have you ever received a blood transfusion or clotting factor treatment?			
Have you received growth hormone of human origin (before 1987)?			
Have you received an organ or tissue (duramater, cornea or other)?			
Have you or any related suffer or have suffered spongiform encephalopathy (Creutzfeldt-Jakob disease)?			
Have you had or have infection with human T-lymphotropic virus (HTLV-I/II)?			
Have you ever been diagnosed with autoimmune disease?			
Baby history			
Is your baby in good health?			
Has he/she had any problem or disease?			
How well did the paediatrician find them during last visit?			
Have you done any analysis on the child?			
Birthplace of the maternal grandmother			
Birthplace of the maternal grandfather			
Birthplace of the paternal grandmother			
Birthplace of the paternal grandfather			
Obstetrical and consanguinity history			
Was this your first son/daughter?			
If no, how many children do you have?			
You and father's child are related (cousins or other relation of consanguinity?)			
Do you have any question or doubt?			

Conclusions:

Reviewed by (Name and Signature) ______________________________

*Transmissible spongiform encephalopathies transmission risk should be considered in persons who lived in the UK between January 1980 and December 1996 for longer than 6 months (for countries other than UK)

Source: adapted from the *Concordia Program* (Barcelona Cord Blood Bank)

Appendix 31. Data collection / Cord blood donor

Preprinted Label | *CB Label* | *Mother donor label*

COLLECTION CENTER:

Donor name: ID:

Geographical Origin

Mother: ☐Spain ☐EU ☐Europe, other ☐Maghreb ☐Subsahara ☐Asia ☐Latinamerica ☐Others:

Father: ☐Spain ☐EU ☐Europe, other ☐Maghreb ☐Subsahara ☐Asia ☐Latinamerica ☐Others:

Travel to endemic areas : ____

INCLUSION QUESTIONNAIRE	**YES**	**NO**
Infectious transmissible diseases	☐	☐
Transmissible genetic disorders found in newborn father, mother and/or siblings	☐	☐
Severe maternal anemia (Hgb< 7g/dL)	☐	☐
Fever (Temperature > 38ºC) (with suspected infection)	☐	☐
Hemolytic disease of the newborn	☐	☐
High-risk delivery	☐	☐

T P A L

Obstetrical history*: ☐☐☐☐

**TPAL (T: term births; P: preterm births <37 weeks; A: abortions <20 weeks; L: living children)*

Medical background of the mother: ____

Medical background of the father: ____

Check if : ☐ pregnancy from egg donation ☐ and/or sperm donation

Negative viral serology during pregnancy for : ☐ HBV ☐ HCV ☐ HIV ☐ Chagas

Donor date of birth : ____________ Age : ________ Weeks of gestation: __________

Delivery type : ☐ Eutocic ☐ Instrumental ☐ Programmed cesarean ☐ Urgent cesarean

Time for cord clamping : _________seconds APGAR: minute 1 _____ minute 5 _____

Drugs administered during childbirth: ____

Incidents, adverse effects detected in the donor: ____

Date : _____/_____/_____ and time _____:_____ of CB collection. Gender: __________ Weight: ____________gr.

Problems detected in the newborn: ____

TRAINED COLLECTOR

ID:

Surname:

Name:

E-mail :

Sign.

SUMMARY OF DISEASES THAT CONTRAINDICATE A DONATION

ADDICTION TO NON-LEGAL DRUGS: Parenterally excludes the donor even if it was only once. Addiction to other drugs, including alcohol, excludes because the donor may have altered the faculties to donate full consent.

ANEMIA: Homozygous or heterozygous father or mother for hemoglobinopathies or hereditary enzymopathies.

SEVERE ECLAMPSIA.

COAGULOPATHIES: Father to mother with hereditary coagulation problems that require treatment and that can be transmitted by the progenitor cells (example: platelet disorders).

DEPRESSION, MANIC-DEPRESSIVE PSYCHOSIS NOT TREATED WITH REGULARITY, DEMENTIA: Not in a position to donate a consent with full autonomy.

HEMOCHROMATOSIS, CIRRHOSIS, WILSON DISEASE.

HEPATITIS, except Hepatitis A, CMV and EBV after 6 months of healing. The sexual partners of people with hepatitis B or C will not be able to donate up to 4 months after the last sexual contact. People who live together (direct domestic contact), including the vaccinated, with carriers of the Hepatitis B virus can not donate up to 4 months after the end of the coexistence. People who have received transfusions can not donate until 4 months.

INFERTILITY (IN TREATMENT): If they have been treated with gonadotropins of pituitary origin before 1987.

AUTOIMMUNE DISEASE: Reumatoid Arthritis, Behcet disease, esclerodermia, amyotrophic lateral sclerosis, multiple sclerosis and its variety Devic disease, spondyloarthropaty, fibromyalgia, reumatic fever, Wegener granulomatosis, Systemic lupus erythematosus polymyositis dermatomyositis, chronic inflammatory demyelinating polyradiculoneuropathy, extensive psoriasis in treatment with Tigasón or with arthritis, immune thrombocytopenic purpura, sarcoidosis, Chronic Fatigue Syndrome, Guillain-Barré syndrome, Sjögren syndrome, systemic vasculitis, vitíligo, Type I diabetes, Reiter syndrome, vasculopathies.

CNS DISEASES: Neurodegenerative diseases, neurofibromatosis, Parkinson.

DERMATOLOGICAL DISEASES: Dermatitis herpetiformis, bullous dermatitis, polymorphic drug erythema, Kaposi syndrome, urticaria pigmentosa, mycosis fungoides, cutaneous leukosis, Sézary's disease.

CREUTZFELDT-JAKOB DISEASE: Diseases suspected of being caused by viruses or prions must be excluded. Definitive exclusion of pituitary hormone receptors of human origin (those administered before 1987). Stay in the United Kingdom: Who excludes people who have lived in the United Kingdom for more than 12 months during the period 1980-1996 on a continuous or intermittent basis. The United Kingdom consists of: England, Wales, Scotland, Northern Ireland, Isle of Man and Channel Islands. The Republic of Ireland (Eire) is not part of the United Kingdom.

NEOPLASTIC HEMATOLOGICAL DISEASES, DEPOSIT AND GENETIC DISEASES.

KIDNEY DISEASES: Chronic renal failure.

ONCOLOGICAL DISEASES: Neoplasms of any type except carcinoma in situ, provided that it is not receiving treatment or has unhealed wounds.

LUNG DISEASES: Sarcoidosis.

THYROID DISEASES: Treatment with radioactive iodine excludes up to 6 months after completion. Exclusion if it is a neoplasm or is part of a multisystem disease. Outside of the above situations, it can be accepted as a donor even if it is being treated with thyroxine.

TROPICAL DISEASES: Donors returning from a trip through tropical countries on risk should not be accepted as donors until after 6 months, and provided they have been free of disease during this period.

MYASTHENIA/MYOPATHY.

INFECTIOUS DISEASES: Donors with a history of tuberculosis or malaria will be excluded for 5 years after the cure. They will be excluded for 2 years after the cure Brucellosis, Q fever (negative serology), Rheumatic fever and Osteomyelitis. After an episode of fever> 38 ° C, potential donors will be excluded for at least 2 weeks from the date of clinical recovery. The history of hepatitis C, Hepatitis B, AIDS, visceral Leishmaniosis (Kala-azar), Babesiosis, Chagas disease, and infection per HTLV-I / II are cause for exclusion. The history of measles, mumps, rubella and varicella excludes up to 4 weeks of cure. In case of Infectious Mononucleosis, the exclusion will be 6 months.

Source: adapted from the *Concordia Program* (Barcelona Cord Blood Bank)

Appendix 32. Informed legal consent for cryopreserving and storing semen from a minor

Date: ________________________ History Nº: __________________

Mr. *(name and surname of father)*____
________________________________ YEARS OLD, ID /PASSPORT Nº______________________
AND RESIDENT AT ____

Mrs. *(name and surname of mother)*____
________________________________ YEARS OLD, ID /PASSPORT Nº______________________
AND RESIDENT AT ____

Mr./Mrs. *(name and surname of child)* ____
________________________________ YEARS OLD, ID /PASSPORT Nº______________________
AND RESIDENT AT ____

Due to the present circumstances of the under age patient (illness, intervention, or medical treatment that may cause sterility, and/or the advisability of preserving a sample of his semen for use in an assisted reproduction treatment), we wish to cryopreserve (freeze) a sample of his semen, which, through this act, we put at the disposal of the clinic so that it may be used at a later date.

For these purposes, we, and in particular the minor, who is intellectually and emotionally capable of understanding the scope of the circumstances, have been informed of the following dispositions: *Law 14/2006 of 26th May on Assisted Reproduction Techniques* and Law 41/2002, of November 14th, governing the independence of the patient and the rights and obligations to clinical information and documentation.

The cryopreserved semen can be kept in authorised gamete banks, during the lifetime of the male to whom it belongs.

The present commitment to preserve the cryopreserved semen is agreed upon for a period of ____ years, during which IVI is committed to adequately maintaining the samples. On expiry of the period agreed upon, we agree to pay the annual amount that we have been informed of through the correspondent economic information, in order to cover maintenance and preservation costs.

Signature *Signature* *Signature*

Should we be interested in possessing the cryopreserved samples, we are committed to contacting IVI to inform them of our intentions. If we do not inform them of our desire to maintain the samples for more time, IVI will understand that we are no longer interested in maintaining them, the cryopreservation and storage commitment, as well as all the other obligations that IVI has assumed, then being automatically resolved, so resulting in the destruction of the sample.

Furthermore, we are committed to informing IVI of any change of address that we, or the minor, may make in the future for the appropriate purposes.

Finally, we have been informed of the absence of risk to the gametes, resulting from their cryopreservation (with the exception of catastrophe or other justified causes outside the control of this centre) and of the economic cost of their cryopreservation and storage.

We (and in particular the minor) have understood the explanations that have been provided to us in clear and simple language and the physician who has attended to us has allowed us to make as many questions as we like and has clarified all the doubts that we have expressed.

The information has been provided to us in sufficient time for us to think it over calmly and to reach a decision freely and responsibly, and it has been communicated to us 24 hours before standard procedure.

I have been informed that pursuant to the Royal Decree 9/2014, following blood collection, serum shall be stored for a specific period of time as provided in the applicable regulations.

We also understand that at any moment and without the need for any explanation, we may revoke the consent which we are now presenting.

We, therefore, declare ourselves to be satisfied with the information received and that we understand the scope and risks of the treatment.

Signature *Signature* *Signature*

Authorization:

We authorize the Reproduction Unit staff to cryopreserve the semen sample that, through this act, we put at the disposal of the clinic, from our son Mr. ________________________________, ____ years old, ID Nº ______________ for the purposes referred to and during the maximum time stated.

In ______________________ Date: ____

Parent´s signatures *Patient´s signature*

Laboratory director´s signature

This Consent form must be signed by the patients on ALL pages and on both sides.

"CONSENT REVOCATION": *For the exercise of patients' rights and for the revocation of this consent, patients may fill in a form requiring the enforcement of such rights at any time during the procedure. This fact will be reflected in the medical history".*

Informed consent declaration (Spanish Fertility Society – SEF) model

"REPRODUCCIÓN HUMANA ASISTIDA, PROTOCOLOS DE CONSENTIMIENTO INFORMADO DE LA SOCIEDAD ESPAÑOLA DE FERTILIDAD", Editorial Comares año 2002.

Human Assisted Reproduction – Informed Consent Models (Spanish Fertility Society Model, 2002).

1 # Appendix 33: Decellularisation
2

3 Several procedures can be applied to decellularise human material for clinical application, but the
4 chosen method should take into account the final specific clinical use of the scaffold and the specific
5 properties needed for this application, since the preserved properties may differ, depending on the
6 method used [1, 2]. Clinical-grade extra-cellular matrix (ECM) may be used to preserve the intrinsic
7 structural and biological properties of the substances of human origin, while removing cells, cellular
8 debris and alloantigen (to minimise immunogenicity). It is also important to achieve the elimination of
9 toxins. However, it must be acknowledged that any decellularisation process carries a certain degree of
10 ECM denaturation.
11

12 ## Decellularisation agents
13 As ECM properties vary between tissues and organs, the main properties to safeguard must be clearly
14 identified and determined in order to choose the correct decellularisation protocol. There are three
15 general methods used to decellularise tissues or organs: physical, biological (enzymatic) and chemical
16 [3, 4]. Each of these methods has a different mode of action and effects on the ECM [1, 5] (see Table
17 33.A), which can be critical for the functionality of the final decellularised ECM. These methods are
18 usually combined to ensure complete decellularisation and can be also combined with different
19 techniques to improve their effectiveness (see Table 33.B).
20 Aside from the selected decellularisation agents, the efficiency of tissues or organs
21 decellularisation will also depend on the intrinsic tissue properties, such as the specific cell density,
22 thickness, compaction and lipid content. The maintenance of each scaffold's mechanical properties is
23 directly related to the maintenance of one or more ECM components. For example, the structural 3D
24 maintenance is related to structural molecules such as collagens, elasticity depends on elastic fibres [6-
25 9] and tensile strength is associated with structural proteins and chondroitin sulphate, while the ability
26 to resist compression is also associated with structural proteins conformation and hyaluronic acid [10].
27

28 **Table 33.A. Techniques used to apply decellularisation agents**

Technique	Advantages	Disadvantages
Perfusion	Facilitates chemical exposure and removal of cellular material	Pressure associated with perfusion can disrupt ECM
Pressure gradient across tissue	Facilitates chemical exposure and removal of cellular material	Pressure gradient can disrupt ECM
Supercritical fluid	Pressure can burst cells. Facilitates chemical exposure and removal of cellular material.	Pressure necessary for supercritical phase can disrupt ECM
Agitation	Can lyse cells. Facilitates chemical exposure and removal of cellular material.	Aggressive agitation or sonication can disrupt ECM

29

30 Physical methods (such as agitation, pressure, freeze/thaw steps, sonication etc.) can be applied
31 but have limited efficacy and should be carefully evaluated to assess any possible damage to the ECM.
32 Usually, they are combined with chemical and enzymatic methods. For the maintenance of ECM
33 structure, ionic detergents could be the optimal choice, and enzymatic or alkaline-acid methods should
34 be avoided, because the damage of the collagen can be limited with time and temperature using an ionic
35 detergent, but the disruption provoked by enzymatic or alkaline-acid methods is highly aggressive. This
36 consideration is valid for the preservation of any protein. Osmotic buffers are a milder method to obtain
37 decellularised ECM, but they are slower and cannot penetrate into thicker, compact organs. For dense

tissues or intact organs, detergents can help buffers to penetrate, but they will affect the protein ultrastructure due to disruption of protein–protein interactions. Furthermore, any residual detergents can have cytotoxic effects and it is imperative to assure their elimination.

Enzymatic treatment should be used to remove specific proteins. However, this technique may cause the unspecific digestion of desired constituents and may not be sufficient for a complete decellularisation of the entire tissue. Alcohols and other solvents can remove lipids very efficiently, but they may also crosslink proteins and modify the ECM ultrastructure. The balance between lipid removal and crosslinking will be determined by the time and temperature of the treatment with these solvents.

Table 33.B. Modes of action and the effect of different decellularisation agents

Method	Mode of action	Effects on ECM
Physical		
Snap freezing	Intracellular ice crystals disrupt cell membranes	Rapid freezing can disrupt or fracture ECM
Mechanical force	Pressure can burst cells, and tissue removal eliminates cells	Mechanical force can damage the ECM
Mechanical agitation	Cell membrane lysis; facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Non-thermal irreversible electroporation	Selective damage on cell membrane while sparing the other tissue components	ECM preservation and produces a functional recellularisable scaffold
Biological (enzymatic)		
Trypsin	Cleaves peptide bonds on the C-side of Arg and Lys	Prolonged exposure can disrupt ECM structure; removes laminin, fibronectin, elastin and glycosaminoglycans (GAG)
Endonucleases	Catalyse the hydrolysis of the interior bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Exonucleases	Catalyse the hydrolysis of the terminal bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Chemical		
Alkaline/acid	Solubilises cytoplasmic components of cells; disrupts nucleic acids	Removes GAG
Hypo/hyperosmotic solutions	Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively remove cellular remnants
Alcohols	Dehydrate and lyse cells. Lipid removal	Fixative properties, protein precipitation and ECM ultrastructure damage
Other solvents (acetone)	Lipid removal	Fixative properties and ECM ultrastructure damage
EDTA/EGTA	Chelating agents that bind divalent metallic ions, thereby disrupting cell adhesion to ECM	Typically used with enzymatic methods
Non-ionic detergents		
Triton X-100	Disrupt lipid–lipid and lipid–protein interactions, while leaving protein–protein interactions intact	Mixed results; efficiency dependent on tissues; removes GAG
Ionic detergents		
Sodium dodecyl sulfate (SDS)	Solubilise cytoplasmic and nuclear cellular membranes; tend to denature proteins	Removes nuclear remnants and cytoplasmic proteins; tends to disrupt native tissue structure, remove GAG and damage collagen

Sodium deoxycolate		More disruptive to tissue structure than SDS and GAG removal
Triton X-200		Achieves efficient cell removal when used with zwitterionic detergents
Zwitterionic detergents		
3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)	Exhibit properties of non-ionic and ionic detergents	Efficient cell removal with ECM disruption similar to that of Triton X-100
Sulfobetaine-10 and 16		Achieves cell removal and mild ECM disruption with Triton X-200
Tri(*n*-butyl)phosphate	Organic solvent that disrupts protein–protein interactions	Variable cell removal; loss of collagen content, although effect on mechanical properties is minimal

In conclusion, specific combinations of mild physical, biological and chemical methods, along with the type of administration, should be tested, validated and controlled to achieve the best results for each tissue or organ, with the aim of obtaining a scaffold that has the desired properties needed for a specific clinical application. Moreover, if the scaffold is a starting material for a medicinal product, the manufacturer should refer to pharmaceutical guidelines for the development, validation and controls of its products.

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