Guide to the quality and safety of TISSUES AND CELLS for human application

European Committee (Partial Agreement) on Organ Transplantation (CD-P-TO)

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

2nd Edition

European Directorate for the Quality of Medicines & HealthCare (EDQM)

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Foreword

Founded in 1949, the Council of Europe is the oldest and largest of all European institutions and now numbers 47 member states.[1] One of its founding principles is that of increasing co-operation between member states to improve the quality of life for all Europeans. Within this context of intergovernmental co-operation in the field of health, the Council of Europe has consistently selected ethical problems for study. One of the most important of these ethical issues relates to the non-commercialisation of human substances: blood, organs, tissues and cells.

Transplantation activities at the Council of Europe are co-ordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM). This Directorate is a key European organisation involved in the harmonisation, co-ordination, standardisation,

1 Albania, Andorra, Armenia, Austria, Azerbaijan, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Republic of Moldova, Monaco, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, San Marino, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, 'the former Yugoslav Republic of Macedonia', Turkey, Ukraine, United Kingdom.

regulation and quality control of medicines, blood transfusion, organ transplantation, pharmaceuticals, pharmaceutical care, consumer health, cosmetics and food packaging.

Transplant medicine and transplantation have progressed during recent decades in a way nobody would have imagined in the preceding years. As with organs, the demand for some transplantable tissues and cells far outweighs the available supply. This has important consequences because human tissues and cells for human application can save lives or restore essential functions. However, as with all transplanted material of human origin, they carry risks of disease transmission that must be controlled by application of scrupulous donor selection and testing criteria, as well as ensuring that comprehensive quality systems are in place.

The first edition of the *Guide to the quality and safety of tissues and cells for human application* was published in 2013. It collated updated information to provide transplant professionals with a useful overview of the most recent advances in the field as well as technical guidance on ensuring the safety and quality of human tissues and cells applied to patients. To increase safety for recipients of human tissues and cells, it is essential that professionals involved in identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood collection centres, tissue establishments processing and storing tissues and cells, and inspectors auditing any of the establishments and organisations responsible for human application have easy access to this information. This Guide aims to support professionals on a practical level and improve the rate of successful and safe clinical application of tissues and cells.

This Guide contains the instructions considered to be the 'minimum standards' that align with relevant European Union (EU) Directives in the field, and provides assistance for those states outside the EU that consider adopting the EU requirements in their legislation. These standards state 'what must be done'. However, this Guide goes beyond these standards by providing additional technical advice, based heavily on good manufacturing practice (GMP) guidelines and on

best practices consistent with current scientific knowledge and expert opinion. It describes background information that should be considered in forming policy decisions, as well as in educational initiatives. This additional information explains the 'why and how'. It also refers to developments that have yet to be incorporated in EU Directives, thereby providing advance information and recommendations regarding technical developments in the field.

Whereas tissue establishments in EU member states are required to comply with legislation derived from EU Directives, this Guide is intended to facilitate ongoing improvements in the donation, procurement, testing, processing, preservation, storage and distribution of tissues and cells through education and provision of non-binding recommendations for EU member states and also for non-EU European countries. At any given time, implementation of these recommendations among member states and individual tissue establishments may vary, and alternative procedures, practices and standards of equivalent safety and quality may be in place.

This Guide has been divided into two parts. Part A contains general requirements applicable to all establishments involved in the donation, procurement, testing, processing, preservation, storage and distribution of tissues and cells that are not manipulated extensively. Part B contains specific guidelines and requirements for the different tissue and/or cell types. The general guidelines of Part A also apply to tissues and cells that have not been mentioned specifically in Part B of the present edition.

In this second edition, many chapters have been improved, and new and important chapters have been added. Most importantly, the field of assisted reproduction is now addressed in detail in Chapter 25 and the topic of fertility preservation is introduced in Chapter 26. The assisted reproduction field is regulated in the EU together with other types of tissues and cells that are applied to patients by Directive 2004/23/EC and its associated technical directives. This field is also included by the World Health Organization (WHO) in its definition of Medical Products of Human Origin (MPHO), which shares a

common need for robust ethical oversight and for safety and quality measures (requirements associated with their common origin in a human donor). A new working group was established with representatives from authorities and the European Society for Human Reproduction and Embryology (ESHRE) to develop these chapters and to carry out an extensive review of the chapters of Part A to ensure that they adequately address this field. It is notable that the generic chapters on topics such as quality management and donor consent, for instance, required minimal adaption to address the field of assisted reproduction. The specific aspects of the field are addressed in detail in Chapter 25 in Part B. As a consequence of the inclusion of assisted reproduction in the guide, Chapter 1 (Introduction) has been updated to include important ethical principles that must be respected in the human assisted fertility field.

A very significant enhancement to the Guide has been the development of a chapter (new Chapter 8) dedicated to the principles of microbiological testing of tissue and cell preparations and their processing environments. Development of Chapter 8 was challenging because in the past the topic has been considered in a disjointed manner, with stakeholders involved in the collection or processing of tissues and cells developing their own principles and practices, and regulatory requirements providing minimal technical detail. The authors of this Guide have worked to achieve a practical and scientifically sound approach to this topic by bringing their prior knowledge and experience of microbiological testing in the field of pharmaceutical manufacture to the discussion. We believe that Chapter 8 is a key addition to this edition, recommending (among other features) the testing methods to be applied in the laboratory and, most importantly, outlining the principles that underpin validation of those methods. Some questions were not fully resolved and will be developed further in future editions.

As the fields of donation and transplantation of tissues and cells evolve, new and more sophisticated technologies will provide opportunities to make tissues and cells safer, or their engraftment more effective. Depending on the degree of complexity of the processing or

the manner in which the tissues or cells are applied to the recipient, some of these tissues and cells are classified in the EU as 'medicinal products'. In many non-EU European countries, this differentiation is not made at a regulatory level, or there is no regulation of the field. Even in EU member states, many tissue establishments are working with tissues or cells that are subsequently sent for manufacture as Advanced Therapy Medicinal Products (ATMP) or are manufacturing those products themselves under the 'hospital exemption' allowed by the Regulations on ATMP. Hence, new chapters have been introduced in the Guide to address this field of cell therapy in particular. Chapter 20 provides a didactic overview of the field by describing the different ways in which cells can be expanded, modified or combined with scaffolds to replace damaged or diseased tissues in the recipient. Chapter 22 describes several types of cells (apart from haematopoietic stem cells) that are rapidly becoming important tools in the fight against disease. These chapters do not attempt to classify such cells or tissues under EU or other regulatory definitions. Instead, they inform readers that they must be fully aware of the regulatory classification of the tissues and cells they are working with, follow GMP, and have a marketing authorisation where they are classified as medicinal products. Chapters of this Guide that relate to recruitment and consent of donors, evaluation and testing of donors, as well as procurement of tissues and cells apply in full to the tissues and cells donated for any clinical application.

A new 'place-holder', Chapter 23, has been added and provides general principles to be considered if banking adipose tissue, an activity that is showing promise for patients with scarring due to tumour removal or burns. Chapter 24 (Other substances of human origin) introduces several other substances obtained from humans for autologous or allogeneic use: human breast milk, faecal microbiota, teeth/dental pulp, platelet-rich plasma, platelet-rich fibrin and serum eye drops. The regulatory status of these substances in most countries is unclear, but their human origin and the processes applied to procure, process and preserve them are analogous to those that apply for the tissues and cells described in this Guide (particularly in Part A). Hence, they

have been added as a means of providing a generic quality and safety framework for healthcare professionals treating patients with these substances.

Other enhancements to the Guide have been division of the former chapter on identification of potential donors, consent and evaluation into two new, more comprehensive chapters: Chapter 3 (Recruitment of potential donors) and Chapter 4 (Donor evaluation). Chapter 5 (Donor testing) now includes a detailed section that describes principles for validation of screening assays for infectious diseases used for testing blood from deceased donors, and considers differences in test results obtained by various types of testing laboratories (hospital-based versus reference laboratory). Chapter 7 (Processing and storage) has been updated to cover more detailed instructions on the requirements of processing facilities as well as monitoring of such facilities (viable and non-viable particles). The text has also been bolstered with more detailed information on the requirements of the cleaning of facilities, storage and exceptional release. Chapters 9 (Distribution and import/export) and 13 (Traceability) include the new EU requirements for the import and coding of tissues and cells. Finally, all of the chapters have been revised thoroughly to update their contents with the most recent advances in the field.

Throughout this Guide, use of the word 'must' indicates mandatory compliance in alignment with EU Directives, whereas use of the word 'should' indicates recommended compliance in accordance with good practice. In addition, unless otherwise stated, the guidelines apply only to human tissues and cells intended for transplantation or clinical use (including insemination and fertilisation). Tissues and cells used for 'basic' research do not fall under the scope of the present Guide.

Two working groups have contributed to the elaboration of this Guide. Working group TO055 helped to update the first edition and their work was co-ordinated by Deirdre Fehily (Italy) and Esteve Trias (European Association of Tissue Banks [EATB]). Working group TO056 drafted the new chapters on 'Assisted reproductive technolo-

gies' and 'Fertility preservation', and their work was co-ordinated by Anna Veiga (ESHRE) and Carlos Plancha (Portugal). Names of the experts that participated in both groups can be found in Appendix 14 of the Guide. These experts contributed to different aspects of the book and did a tremendous job in reviewing the literature and extracting knowledge from numerous international guidelines, collaborative projects and diverse publications and Internet websites with the aim of ensuring accessibility to all this information.

In addition, other experts contributed in the discussions on various parts of this Guide and should be acknowledged:

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The drafting and publication of the second edition of the Guide was co-ordinated by Marta López Fraga (Scientific Officer in charge of the Council of Europe European Committee on Organ Transplantation [CD-P-TO]) with the assistance of Ahlem Sanchez, David Crowe and Isabelle Vernay. An extended thank you should also be given to Karl-Heinz Buchheit, Head of the Department of Biological Standardisation, OMCL Network & HealthCare (DBO), and Susanne Keitel, Director of the EDQM.

The entire project has been an exceptional combined effort, with extensive discussions dedicated towards the common goal of increasing the safety, efficacy and quality of donation of tissues and cells, as well

as the testing, processing and storage for clinical application of tissues and cells. The final result is this Guide, which constitutes a common European standard, based on the long-standing expertise and knowledge of the EDQM.

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PART A. GENERAL REQUIREMENTS

Chapter 1

Introduction

1.1. Scope and purpose of this guide

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 in an increasing variety of new ways. Many of these developments, such as advances in transplantation therapy or in assisted reproduction technology (ART), 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 people. Some other tissues and cells can be provided only by living donors, as long as this procedure does not 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 procreate.

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 de-cellularised 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 and craniofacial/maxillofacial, dental 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 bones can also be supplied in soft, pliable or injectable forms. If a deceased individual has consented to the use of any part of his/her body for the treatment of others (or their relatives have authorised this after his/her death), many tissues can be put to use to fulfil this wish; bone, heart valves, skin, corneas, ligaments, cartilage, connective and adipose tissue, glands and nerves can all be used for therapeutic purposes.

In contrast, amniotic membranes from the placenta, parathyroid tissue, and skull bone are given by living persons. Additionally, heads of femurs 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 cells can be donated (some during life and some after death) and submitted to different degrees of manipulation before application in humans. Examples include haematopoietic stem cells (e.g. bone marrow, peripheral blood stem cells, umbilical cord blood), somatic cells (e.g. peripheral blood cells, keratinocytes, chondrocytes, hepatocytes), mesenchymal stem cells, and limbal stem cells. Oocytes, sperm, ovarian, testicular tissue and embryos can be used in ART procedures to achieve pregnancy.

This is the second 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. 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. Human tissues and cells for clinical applications represent a special class of basic essential healthcare products, as well as being the potential starting material for much more complex products in the future. All material of human origin carries risks of disease transmission that must be controlled by application of scrupulous criteria of donor selection (including 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 reflects 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 (or are in the process of being) 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. 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 operators 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 and distribution, imports and exports;

d. there are programmes for vigilance and surveillance of adverse outcomes;

e. there is monitoring and reporting of donation, processing, storage, distribution and import and 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 terms, such as 'Regulatory Authority', 'Regulatory Agency' or, in the EU, 'Competent Authority', are equivalent to it.

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 is developing artificial tissue that should alleviate the shortage of tissues available for transplantation. These forms of 'basic' research using human tissue still have an ultimately therapeutic goal in mind. However, as important as all these possibilities are, this Guide will only cover tissues and cells used for current therapeutic purposes.

Finally, a glossary of terms is provided in the Appendices.

This book is the result of the collective effort and expertise gathered by the members and observers of the European Committee of Experts on Organ Transplantation (CD-P-TO) and the Ad hoc Tissues and Cells Expert Groups (see Appendices). Unless otherwise indicated, 'member states' applies to member states of the Council of Europe.

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

1.2. Brief history of tissue and cell transplantation and banking

The best documented accounts of early transplants deal with skin transplantation, though the success or failure of these procedures has not been well documented. The first reliable account is that of the Indian surgeon Sushruta in the 2nd century BC, who used auto-grafted skin transplantation for a nose reconstruction (rhinoplasty). Centuries later, the Italian surgeon Gasparo Tagliacozzi carried out successful skin auto-grafts, but he consistently failed with allografts, offering the first suggestion of rejection centuries before that mechanism could possibly be understood. He attributed it to the 'force and power of individuality' in his 1596 work *De Curtorum Chirurgia per Insitionem*. Orthopaedic surgeons refer to the origin of their discipline as 1668 when Job van Meekeren reported on the grafting of bone from a dog's skull to correct a defect in a soldier's cranium. It was not until 1869 that the first completely documented fresh human skin allograft was carried out by the Swiss surgeon Jacques Reverdin.

The first successful human corneal transplant, a keratoplastic operation, was carried out in 1905 by Eduard Zirm at Olomouc Eye Clinic (Czech Republic). Pioneering work in the surgical technique of transplantation was made in the early 1900s by the French surgeon Alexis Carrel, together with Charles Guthrie, who developed techniques for suturing arteries and veins. Their skilful anastomosis operations and new suturing techniques laid the groundwork for later transplant

surgery, and Alexis Carrel won the 1912 Nobel Prize in Physiology or Medicine for his work in the field. Major steps in skin transplantation occurred during the First World War, notably through the work of Harold Gillies in Aldershot, UK. Among his advances was the tubed pedicle graft, which maintained a fleshy connection from the donor site until the graft established its own blood supply.

Bone is the oldest tissue transplant on record and the most common tissue transplanted today. The first bone transplant recorded in modern times occurred in Scotland in 1878 when Sir William Macewen removed an infected humerus from a 12-year-old boy and replaced it with three allografts from an amputated tibia from another child with rickets. In 1907, Erich Lexer in Berlin developed a procedure to remove a whole knee joint from an amputee in one operating room and transport the 'warm' graft to an adjacent operating room for immediate transplant into the recipient. Five years later, Alexis Carrel's work predicted the storage of tissues for future transplantation, and surgeons began to use bones and developed their own 'bone banks'. These pioneers included Inclan in Cuba, Bush, Wilson and Hibbs in the USA, Hult working in Sweden, Judet in France, and Klen in what was then Czechoslovakia. Most of these early bone banks were simply refrigerators and, later, freezers, but greater sophistication was developed by bone banks in Leeds (UK), Berlin, Athens and Warsaw. When long-term freezer storage of long bones became feasible, limb-sparing surgery using this type of bone allograft to avoid amputations in the treatment of malignant skeletal tumours became popular. Burrwell (UK), Parrish and Mankin (USA) and Ottolenghi (Argentina) published their results. The orthopaedic profession realised that if very large segments of bone could be transplanted successfully, smaller segments could also be used. This resulted in a very large increase in the use of bone allografts. Tissue storage methods were developed further during the 1950s by Hyatt at the US Navy Tissue Bank in Bethesda, Maryland, where they adapted methods of lyophilisation from the food preservation industry and applied the process to the preservation of bone and skin, which could then be easily stored, transported and reconstituted for use when needed. This method of preservation

allowed bone to be stored and transported easily without any electrical or mechanical requirements, and has had a profound effect on the availability and use of bone allografts. By the end of the 1990s, use of musculoskeletal tissue allografts (i.e. bone, cartilage, soft tissue) had become commonplace in many clinical areas. Similarly, the first deceased donor eye bank was established in Odessa using eyes (packed in storage medium in glass containers) sent by rail from a trauma centre in Moscow.

The first recorded cardiac valve transplantation was carried out by Gordon Murray in Toronto, 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 World War II, many scientists began to explore ways of protecting humans from irradiation. 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 transplanted 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 leucocyte 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 immune 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 a 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 stem cell donors has increased all over the world, with more than 24.5 million donors now registered, including 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 living donor pancreatic islet transplant from a 56-year-old woman to her 27-year-old diabetic daughter re-

sulted 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 (PGD) [8], and clinical management (such as improving methods for ovarian stimulation and transvaginal oocyte retrieval), thereby leading to a considerable increase in the use of ART. Today, more than 5 million babies have been born worldwide through ART.

1.3. European Committee on Organ Transplantation, the European Directorate for the Quality of Medicines & HealthCare and the Council of Europe

The Council of Europe, based in Strasbourg (France), is an international organisation that promotes co-operation between all European countries in the areas of human rights, democracy, rule of law, culture and public health. After the Third Conference of European Health Ministers on the Ethical, Organisational and Legislative Aspects of Organ Transplantation [9] held in Paris in 1987, the Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation (SP-CTO) was created. This Committee consisted of experts in different aspects of transplantation: immunologists, surgeons, physicians, as well as co-ordinators and representatives from organ-sharing and organ-procurement organisations. In 2007, the Secretariat responsible for activities related to organs, tissues and cells was transferred to the European Directorate for the Quality of Medicines & HealthCare (EDQM) of the Council of Europe [10], and the newly appointed CD-P-TO took over as the steering committee [11].

Today, the CD-P-TO is composed of internationally recognised experts from Council of Europe member states, observer countries,

the European Commission, the WHO, representatives from the Committee on Bioethics of the Council of Europe (DH-BIO) and several non-governmental organisations. It actively promotes the non-commercialisation of organ donation, the fight against organ trafficking, the development of ethical, quality and safety standards in the field of organs, tissues and cells, and the transfer of knowledge and expertise between member states and organisations.

1.4. Recommendations and regulations in the field

1.4.1. Council of Europe

Within the framework principle of sharing knowledge through international co-operation, the Council of Europe has established widely recognised recommendations and resolutions in the field of transplantation covering the ethical, social, scientific and training aspects of the donation and transplantation of organs, tissues and cells [12]. Whereas agreements and conventions are binding on the states that ratify them, resolutions and recommendations are policy statements to governments that propose a common course of action to be followed.

The Council of Europe *Convention for the Protection of Human Rights and Fundamental Freedoms* (European Treaty Series, No. 5) [13] is an international treaty to protect human rights and fundamental freedoms in Europe. It was drafted in 1950 by the then newly formed Council of Europe and entered into force on 3 September 1953.

The *European Agreement on the exchange of therapeutic substances of human origin* (European Treaty Series, No. 26) [14], signed in Paris on 15 December 1958, aims to provide mutual assistance with respect to the supply of therapeutic substances of human origin.

The *European Agreement on the exchange of tissue-typing reagents* (European Treaty Series, No. 84) [15], signed in Strasbourg on 17 September 1974, lays the groundwork for the development of mutual assistance in the supply of tissue-typing reagents and establishment of joint rules between signatory parties. The signatory parties undertake to make reagents available to other parties who are in need of them, by

the most direct route, subject to the condition that no profit is made on them, that they must be used solely for medical and scientific purposes and are free of import duties. The *Additional Protocol* (European Treaty Series, No. 89) [16], opened for signature on 24 June 1976 and which entered into force on 23 April 1977, provides for the accession of the European Community to this Agreement.

The *Oviedo Convention: Protection of human rights and dignity of the human being with regard to the application of biology and medicine* (European Treaty Series, No. 164) [17], opened for signature on 4 April 1997 and which 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 that the body and its parts, as such, give rise to financial gain.

This latter Convention was extended further 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) [18], 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 guarantee, without discrimination, respect for his/her integrity and other rights and fundamental freedoms with regard to 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 and its Explanatory Report* (European Treaty Series,

No. 197) [19], opened for signature in Warsaw on 16 May 2005 and which came into force on 1 February 2008, addresses the trafficking of human beings for the purpose of organ removal.

The *Joint Council of Europe/United Nations Study on trafficking in organs, tissues and cells and trafficking in human beings for the purpose of the removal of organs* [20], 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 transplantation-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* [21] and its *Explanatory Report* [22], 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 on Harmonisation of legislations of member States relating to removal, grafting and transplantation of human substances* [23];
- *Recommendation No. R (94) 1 of the Committee of Ministers to member States on human tissue banks* [24];

- *Recommendation No. R (98) 2 of the Committee of Ministers to member States on provision of haematopoietic progenitor cells* [25];
- *Recommendation Rec (2004) 8 of the Committee of Ministers to member States on autologous cord blood banks* [26];
- *Recommendation Rec (2006) 4 of the Committee of Ministers to member States on research on biological materials of human origin* [27].

Monitoring of practices in member states has become an evident need for the sake of transparency and international benchmarking. Keeping this goal in mind, since 1996 the EDQM/Council of Europe has published *Newsletter Transplant* [28], which is co-ordinated by the Organización Nacional de Trasplantes (ONT) in Spain. This publication summarises comprehensive data provided by national focal points designated by governments on donation and transplantation activities, management of waiting lists, organ-donation refusals, and authorised centres for transplantation activities. *Newsletter Transplant* provides information from ≈70 countries, including Council of Europe member states, observer countries and observer networks (e.g. Iberoamerican Donation and Network Council on Organ Donation and Transplantation, Mediterranean Network). The *Newsletter Transplant* database is connected with other international projects on data collection (e.g. WHO Global Observatory on Organ Donation and Transplantation, Eurocet database) to avoid duplication of efforts. *Newsletter Transplant* has evolved into a unique official source of information that continues to inspire policies and strategic plans worldwide.

The Council of Europe also produces other guidelines, including this second edition of the *Guide to the quality and safety of tissues and cells for human application*, the 5th edition of the *Guide to the quality and safety of organs for transplantation* and the 18th edition of the *Guide to the preparation, use and quality assurance of blood components.*

1.4.2. World Health Organization

In 1987, the 40th World Health Assembly, concerned about the trade for profit in human organs, initiated preparation of the first *WHO Guiding principles on transplantation*, endorsed by the Assembly in 1991 in resolution WHA 44.25 [29]. These Guiding Principles have greatly influenced professional codes and practices, as well as legislation, around the world for almost two decades. After a consultation that took several years, the 63rd World Health Assembly adopted resolution WHA 63.22 [30] on 21 May 2010, which endorsed the updated *WHO Guiding principles on human cell, tissue and organ transplantation* [31] and called on WHO member states to: implement these guiding principles, promote voluntary and unremunerated donation, oppose trafficking, and promote transparent and equitable allocation. It also urged its members to strengthen oversight, to collect and publish activity data, including adverse events and reactions, and to implement globally standardised coding. These guidelines are intended to provide an orderly, ethical and acceptable framework for the acquisition and transplantation of human cells, tissues and organs for therapeutic purposes.

The World Health Assembly adopted resolution WHA 57.18 [32] in 2004, which urged WHO member states 'to take measures to protect the poorest and vulnerable groups from transplant tourism and the sale of tissues and organs, including attention to the wider problem of international trafficking in human tissues and organs'. Subsequently, the *Declaration of Istanbul on organ trafficking and transplant tourism* [33] was adopted in 2008, as an initiative of The Transplantation Society (TTS) and the International Society for Nephrology (ISN). The Declaration emphasises that organ trafficking and transplant tourism should be prohibited because they violate the principles of equity, justice and respect for human dignity. The Declaration asserts that because transplant commercialism targets impoverished and otherwise vulnerable donors, it leads inexorably to inequity and injustice and should also be prohibited. Organ trafficking, transplant tourism and transplant commercialism were defined by the Declaration by providing principles of practice based on those definitions. The

Declaration of Istanbul distinguishes transplant tourism from travel for transplantation. Travel for transplantation is the movement of organs, donors, recipients or transplant professionals across jurisdictional borders for transplantation purposes. Travel for transplantation becomes transplant tourism if (1) it involves organ trafficking and/or transplant commercialism or (2) if the resources (organs, professionals and transplant centres) devoted to providing transplants to patients from outside a country undermine the country's ability to provide transplant services for its own population.

Robust bi-directional donor–recipient traceability is a prerequisite to achieving effective vigilance and surveillance worldwide. For this reason, Resolution WHA 63.22 [30] also urged WHO member states to collaborate in collecting data (including adverse events and reactions) in addition to implementation of globally consistent coding systems. The Notify project was a specific follow-up action that was led by the WHO to promote the sharing of information on adverse incidents for improving safety and efficacy [34].

As a result of resolutions WHA 57.18 and WHA 63.22 (which requested that global data on the practice, safety, quality, efficacy and epidemiology of transplantations be collected in the WHO member states that have transplantation programmes), an international watchdog on transplantation was set up as a collaborative initiative between the Spanish ONT and WHO, and was termed the Global Observatory on Donation and Transplantation [35]. The universal availability of these data is recognised as a prerequisite for global improvements in demonstrating transparency, equity and compliance, and for monitoring systems in countries. In addition, the data provided also help to give an overview of the legal and organisational aspects in very different settings and countries, which enables the regulating bodies to monitor transplantation activities.

The WHO has also published two *Aide-Mémoires* specifically for the donation and transplantation of tissues and cells [36, 37].

In recent years, the WHO has been promoting use of the term 'medical products of human origin' (MPHO). This category includes

blood, organs, tissues, bone marrow, cord blood, reproductive cells and milk derived from humans for therapeutic use. Use of these MPHO, obtained from living and deceased donors, entails practical, scientific and ethical considerations.

1.4.3. European Union

1.4.3.1. *EU tissues and cells legislation*

Article 168 of the *Treaty on the Functioning of the European Union* [38] (previously Article 152 of the *Treaty of Amsterdam*) gives the EU a mandate to establish high quality and safety standards for substances of human origin, such as blood, organs, tissues and cells.

Acknowledging that the transplantation of human tissues and cells is an expanding medical field that offers important opportunities for the treatment of disease, the EU aims for a common approach to the regulation of tissues and cells across Europe.

The EU Tissue and Cells Directives have created a benchmark for the standards that must be met if carrying out any activity involving tissues and cells for human applications, including gametes and embryos and germinal tissue. The Directives also require that systems be put in place to ensure that all the tissues and cells used in human applications are traceable from donors to recipients and vice versa.

Directive 2004/23/EC [39] of the European Parliament and of the Council of 31 March 2004 applies to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells intended for human use (including reproductive cells used in ART procedures). The Directive introduced obligations on EU member states' authorities, from supervision of human tissue and cell procurement and authorising and inspecting tissue establishments, to ensuring traceability and vigilance and maintaining a publicly accessible register of national tissue establishments. It also lays down provisions regarding donor selection and evaluation (e.g. principles governing tissue and cell donation, consent, data confidentiality) and quality and safety of tissues and cells (e.g. quality management, tissue and cell reception, processing and storage conditions).

Commission Directive 2006/17/EC [40] 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 and procurement procedures and reception at the tissue establishment and requirements for direct distribution to the recipient of specific tissues and cells. Directive 2006/17/EC was amended in 2012 by the Directive 2012/39/EU with regard to certain technical requirements for the testing of human tissues and cells [41].

Commission Directive 2006/86/EC [42] includes traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

In 2015, two new Commission Directives were adopted, one implementing Directive regarding the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells [43] and a second one amending Directive 2006/86/EC, providing detailed requirements on the coding of human tissues and cells [44].

Quality and safety standards for human organs intended for transplantation are laid down in Directive 2010/53/EU and Commission Implementing Directive 2012/25/EU. These detail standards and procedures for information exchange between EU member states regarding human organs intended for transplantation [45, 46].

The EU Directives dictate that EU member states must encourage voluntary and unpaid donations of tissues and cells and must endeavour to ensure that the procurement of tissues and cells is carried out on a non-profit basis. Promotion and publicity activities in support of the donation of human tissues and cells with a view to offering or seeking financial gain or comparable advantage are not allowed. The EU Directives also provide clear mandates as regards the consent of donors and the anonymity of all data collected, and instructs EU member states to adopt measures to ensure data security and prevent unauthorised modifications to files and records.

These Directives do not cover research using human tissues and cells (e.g. *in vitro* research or research in animal models) and do not interfere with the decisions of EU member states on the use or non-use of any specific type of human cells, including embryonic stem cells. Similarly, these Directives do not interfere with provisions of member states defining the legal term 'person' or 'individual'.

The European Commission has supported EU member states in their efforts to implement EU Directives on tissues and cells by providing funding for several projects under the Programme of Community Action in the Field of Health [47]:

- EQSTB (European Quality System for Tissue Banking) focused on four main work packages: (i) identification of the key requirements for tissue banking; (ii) development of a registry to support exchange of tissues; (iii) provision of training programmes, both online and face-to-face, to fulfil the needs of tissue establishment professionals and (iv) development of an audit model and audit guide for tissue establishments, with recommendations for tissue establishments and guidance for auditors.
- EUSTITE (European Standards and Training in the Inspection of Tissue Establishments) [48] developed guidance and training courses for EU Competent Authorities on the inspection of tissue establishments and on vigilance for tissues and cells used in transplantation and in assisted reproduction. The guidance document served as a basis for the guidelines on the implementation of inspection and control measures in the field of human tissues and cells included in Commission Decision 2010/453/EU of 3 August 2010.
- POSEIDON (Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe) provided recommendations for improvements in the safety of unrelated haematopoietic stem cell transplantation, for the optimisation of human stem cell donation policy,

and for promoting equal access to this therapy throughout the EU.

- EUROCET [49] is a platform funded initially by the European Commission but which is now maintained by the Italian National Transplant Centre. It is a publicly accessible registry of tissue establishments and Competent Authorities for tissues and cells in the EU and it collects and publishes annual activity data on donation, processing and human applications of tissues and cells.
- EuroGTP (European Good Tissue Practices) [50] developed a *Good tissue practices guide and Personnel training guidelines* for tissue establishments regarding recovery, processing and preservation of tissues to ensure that all tissue establishments guarantee the highest level of quality and safety of tissues for transplantation. EuroGTP has provided a crucial basis for much of the technical content of this Guide. A strong collaboration between the European Association of Tissue Banks (EATB), which will update and maintain the GTPs as their own standards, and the Council of Europe will be maintained to ensure consistency and development in the light of the most up-to-date scientific knowledge.
- The project SOHO V&S (Vigilance and Surveillance of Substances of Human Origin) [51] addressed the harmonisation of terminology and documentation relating to adverse events and reactions. They aimed to find a consensus on how information should be exchanged between EU member states, the European Commission and third countries to enhance efficient management of incidents involving cross-border distribution of tissues and cells. The project drafted important guidance documents for the EU competent authorities, concerning the detection and investigation of suspected illegal and/or fraudulent activity related to tissues and cells, the communication and investigation of serious adverse events and reactions associated with human tissues and cells, as well as the vigilance and surveillance in

the field of assisted reproductive technologies. The project also prepared a guidance document for healthcare professionals on vigilance and surveillance of human tissues and cells. A training model for Competent Authorities in the investigation and management of vigilance and surveillance of tissues and cells was also provided.

- The joint action ARTHIQS (Good Practice on Donation, Collection, Testing, Processing, Storage and Distribution of Gametes for Assisted Reproductive Technologies and Hematopoietic Stem Cells for Transplantation) [52] was launched in 2014. It is a three-year project that will build institutional and inspection guidelines in assisted reproductive technologies as well as guidelines related to the set-up and regulation of haematopoietic stem cell donor follow-up registries and banking of cord blood.

These projects have strengthened collaboration among health authorities and between these health authorities and the professional associations in the area of tissue/cell transplantation, allowing continuous input from field practice into the regulatory framework.

1.4.3.2. *Other relevant EU legislation*

When human tissues and cells are used in the manufacture of medicinal products which are either a gene therapy medicinal product, a somatic cell therapy medicinal product, or a tissue engineered product, Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products ('ATMP Regulation') applies [53, 54] (see Chapter 22). For such products derived from human tissues and cells, Directive 2004/23/EC and its implementing Directives apply only to their donation, procurement and testing.

When clinical trials include human tissues and cells applied to the human body, beside the quality and safety standards laid down in the Directive 2004/23/EC and its implementing Directives, the EU legislation on clinical trials [55] is applicable.

Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and the free movement of such data [56] must be applied when processing personal data (e.g. data related to donors and recipients).

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

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 also donor-related disease transmission risks. The factors influencing the clinical outcome of transplantation are complex; there is an interaction between two different biological systems, i.e. those of the donor and the recipient. Therefore, when assessing the risk of human application of tissues and cells, the 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.

Some of the most widely used tissues and cells and their benefit for transplanted recipients are listed in Table 1.1.

Table 1.1. Most widely used tissues and cells and the benefits for the transplanted recipients

Tissues and cells	Function	Benefits for the recipient
Bones	Support the body and protect vital organs.	Used to repair or stabilise the spine and other bones damaged from degeneration, trauma, cancer or birth defects. Bone is also used in oral surgery and in the filling of bone cavities or other areas where bone mass has been lost.

Tissues and cells	Function	Benefits for the recipient
Haematopoietic stem cells (bone marrow, peripheral blood stem cells and cord blood)	Haematopoiesis	Used for the treatment of haematological malignancies as well as genetic and autoimmune diseases.
Corneas/eyes	Corneas allow light to enter the eye. Sclera is the white of the eye, which provides structure and support.	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 oral grafts in dental procedures or for the treatment of glaucoma.
Gametes, reproductive tissues and embryos	Generation of a new human being.	
	Used primarily for infertility treatments. However, they can also be applied to couples to avoid the transmission of genetic or infectious diseases, to single women, and to same-sex couples. They can also be stored to preserve fertility.	
Fascia	Fibrous tissue that covers muscles.	Used to repair tendons, muscle, ligaments and deformities.
Heart valves	Direct the flow of blood in the heart.	Used for patients with valve defects, especially in children.
Pancreatic islets	Containing Langerhans cells, which are responsible for insulin production.	As an alternative to pancreatic transplants.
Pericardium	Protective lining around the heart.	Used for replacement of dura mater in the brain and for eye surgery.

Tissues and cells	Function	Benefits for the recipient
Placenta/amniotic membrane	Transfers oxygen and nutrients from the mother to the foetus.	The amniotic membrane is used in burns (to reduce surface inflammation, scarring and pain in surgical applications), in certain types of ulcers, and in eye surgery.
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 reroute blood flow.
Skin	Protects the body against injury, infection and dehydration.	Used for the treatment of burns patients, certain types of ulcers, abdominal wall repairs and reconstructive or plastic surgery.
Tendons	Attach muscle to bone.	For use in joint injuries.

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:

> '… 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 medical case history, travel history, behavioural risks and history of malignancies are necessary to keep the risk of transmission of infections or malignancies to the recipient as low as possible. These parameters 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, pro-

cessing, storage and distribution are likely to function satisfactorily and to reach an acceptable level of safety. The donor selection criteria as well as the processing and preservation conditions 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, donation of tissues or cells that does not meet all the necessary safety or quality requirements may be used for human application into a particular patient. This may occur, for instance, where the transplant is likely to be life-saving and alternative options for treatment of that patient carry a poor prognosis. Similarly, couples undergoing ART 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). 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.

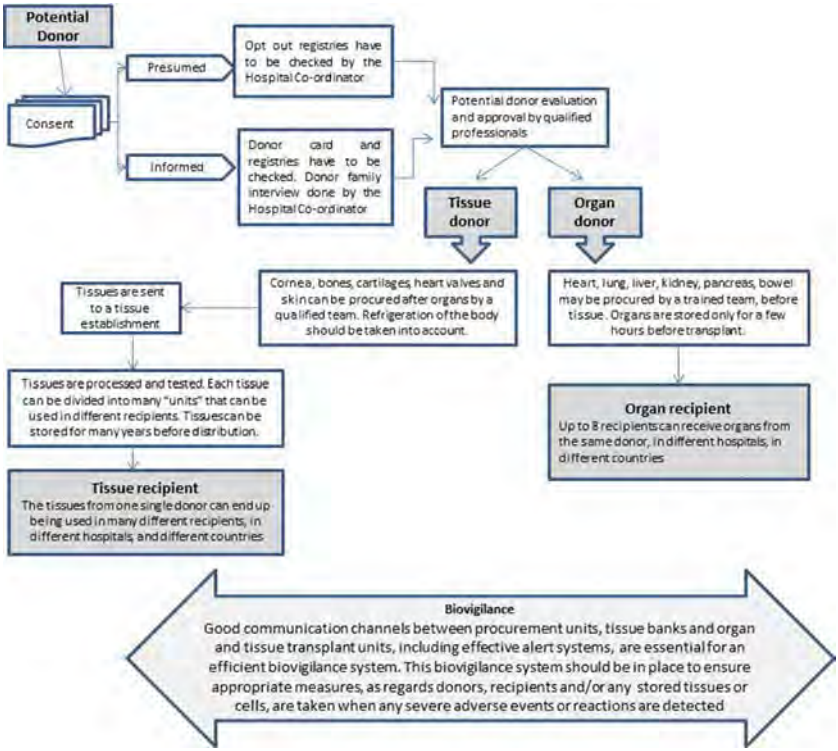
1.6. Process of donation of tissues and cells and application in humans

Donation of tissues and cells and 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 (removal, 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 material may be taken), and the complex chain of inter-

mediaries (people and institutions) in the process of donation and transplantation.

The process of donation of tissues or cells from a deceased donor is, in many respects, quite different from the process in living donors. However, 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.

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



It is appropriate to 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, there is the necessary infrastructure to procure tissues within a given timeframe and process them, appropriate transport services exist to transport tissues in an adequate manner, and surgeons are available to carry out tissue transplantation into the recipient. Similarly, for living donation to be made possible, professionals need to recruit and evaluate potential donors, and adequately trained personnel must carry out the complex processes that will lead to generation of the medical products needed to treat patients.

The histories of the many forms of tissue banking and cell banking highlight the increasingly complicated and interconnected ways in which one person's tissues and cells may be used to help others or themselves. The central role of tissue establishments in modern medicine in providing material for treatment and for research highlights the complicated networks that may now connect the sources and recipients of donated bodily material, and the many intermediaries involved in processing the material, to facilitate its use by the clinical user.

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 often be relatively limited. National and international efforts have focused on 'best 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. Thus, the flows involved between the original 'source' or donor of the material, the amount of processing of the material involved and the commercial nature of some of those transactions are becoming ever more complex.

It is important to emphasise how consideration of policy surrounding donation must increasingly take into account the complex flows and multiple intermediaries involved in the process [57]. Such awareness highlights the central part inevitably played in the donation and subsequent use of bodily material by organisations and organisational structures. These include, for example, the creation of professional roles such as donation and consent 'co-ordinators' and the extent to which they are expected to maximise opportunities for donation, how these professionals approach potential donors and form relationships with them, how well one part of the system links with another and where responsibility is seen to rest, and the way professionals in different fields interact and co-operate with one another. It also points to added complexities in the form of legal agreements, liabilities and obligations that may arise where donated material is transformed, banked or otherwise handled as a commodity by successive intermediaries.

The increasing possibility of using many forms of bodily material to benefit others in medical treatment has brought about increased pressure in member states to meet demand. There is a continual need to recruit new tissue and cell donors to maintain an adequate supply. Shortages of supply may affect particular subgroups of the population more than others because of the need to match material according to immunological criteria or age. 'Demand' for material is inherently variable; as scientific developments make more treatments possible, the demand for that treatment is likely to increase, and the development of alternatives may lead to reduced demand. Public expectations of what medical science can achieve may serve to put further upward pressure on demand.

Talking in terms of 'supply' and 'demand' may resonate with the experiences of many professionals and patients (potential recipients), who are only too aware of the impact of any shortage in supply. This feature is exacerbated in situations in which the requirement for a high degree of matching or phenotypical similarities between donor and recipient calls for recruitment from ethnic minorities and international collaboration. However, at the same time, it may imply a lack of consideration

of the human nature of the source of the material. It is important to emphasise when using these impersonal terms that we are talking about people and people's lives.

1.7. Tissue banks, biobanks and tissue establishments

A 'tissue bank' is a term commonly used to describe an establishment that collects and stores human tissues for either medical research or a medical application/transplantation.

Increased use of tissues and cells for human application and research calls for terminology that will help to distinguish between establishments that collect and store tissues and cells for each of those purposes. Within Europe, the terms currently in use are tissue establishments and biobanks, respectively.

The term 'biobank' is used regularly for repositories storing human biological samples for use in research. Presently, there is not an internationally-agreed definition of biobank, but the term is generally used for organised collections of human biological material (blood, tissues, cells, other body fluids, DNA, RNA, etc.) and associated information stored for one or more research purposes. In its glossary, the Organisation for Economic Co-operation and Development (OECD) defines a biobank as 'a collection of biological material and the associated data and information stored in an organised system, for a population or a large subset of a population' [58]. Several other definitions, as used in various EU legislation/guidelines, are available on the website of the EU-funded project PRIVILEGED (Privacy in Law, Ethics and Genetic Data) [59].

In the USA, the term 'biorepository' is preferred to biobank. For example, according to the glossary of the National Cancer Institute, a biorepository is:

> 'a facility that collects, catalogues, and stores samples of biological material, such as urine, blood, tissue, cells, DNA, RNA, and protein, from humans, animals, or plants for laboratory research. If the samples are from people, medical

information may also be stored along with a written consent to use the samples in laboratory studies' [60].

The term 'tissue establishment' became widely used in Europe following publication of the EU Tissues and Cells Directive 2004/23/EC, which defines it as 'a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells are undertaken. It may also be responsible for procurement or testing of tissues and cells'. In the ART field, the term 'tissue establishment' refers to the laboratories in ART centres or clinics as well as banks of gametes. These centres or clinics often also include clinical units in which the patients are treated. In the context of this Guide, the term 'tissue establishment' will be used and refer to all of the banks, units, centres and clinics mentioned above. The Directive does not cover research using human tissues and cells, so tissue establishments are concerned only with tissues and cells intended for human application. Similarly, the Directive also does not cover the clinical application and practices undertaken in the clinical units of the ART centres.

In the USA, the American Association of Tissue Banks (AATB) uses the term tissue bank for 'an entity that provides or engages in one or more services involving tissue from living or deceased individuals for transplantation purposes. These services include assessing donor suitability, recovery, processing, storage, labelling, and distribution of tissue' [61].

The biobanking field is continuously evolving, and tissue establishments may become interested in collecting samples for research purposes, so the terminology should also be refined to reflect these changes in the future.

In this Guide, it was agreed to use the term 'tissue establishment' and its definition in accordance with Directive 2004/23/EC.

1.8. Quality and safety

High-quality, safe and efficacious procedures are essential for donors and recipients alike. The long-term outcomes of tissue and cell dona-

tion and transplantation should be assessed for the living donor, as well as the recipient, to document benefit and harm.

The level of safety, efficacy and quality of human tissues and cells for human application as health products of an exceptional nature must be maintained and optimised on an ongoing basis. This strategy requires implementation of quality systems that include traceability and vigilance, with adverse events and reactions reported both nationally and for imported/exported human products.

Optimising the outcome of tissue and cell transplantation entails a rules-based process that encompasses clinical interventions and *ex vivo* procedures from donor selection through to long-term follow-up. With the oversight of Health Authorities, transplant and ART programmes should monitor donors and recipients to ensure that they receive appropriate care, including information regarding long-term risks and benefits. Evaluation of information regarding long-term risks and benefits is essential to the consent process and for adequately balancing the interests of donors and recipients. The benefits to both must outweigh the risks associated with donation and transplantation/human application. Donors should not be permitted to donate in clinically hopeless situations.

Locally organised donation, transplant programmes and ART programmes should store details of their activity and follow-up data in national and/or international registries. All deviations from accepted procedures that could increase the risk to recipients or donors (as well as any untoward consequences of donation or human application) should be reported to, and analysed by, the responsible Health Authorities.

Transplantation of human material that does not involve long-term medical care of the recipient may not require active, long-term follow-up; though traceability should be ensured for the anticipated lifetime of the donor and the recipient. Internationally agreed means of coding to identify tissues and cells used in transplantation are essential for full traceability.

In the specific case of ART, traceability should include the outcome of the pregnancy as well as the health of the donor, recipient and newborn. It is of the utmost importance to put the welfare of donors (especially with respect to non-partner oocyte donors) in a central position in determining what constitutes acceptable practice. This requirement might entail additional effort in the context of cross-border reproductive care. All gamete donors should be recorded in national registers, and all centres should participate in the collection of national and international data. ART centres and Health Authorities should collect data on a systematic basis to follow-up the long-term health effects of ART activity, including the health of the donor, recipient and newborn. Good-quality evidence on these effects is essential for appropriate concern to be given to the welfare of oocyte donors in future policies. In addition, there should be a limit to the number of times a woman may donate. Ultimately, the welfare of oocyte donors should underpin any consideration on donation.

1.9. Ethical issues

Human tissues and cells can be derived only from the body of a person – hence the ethical challenges associated with their use. The range of materials described in this Guide makes explicit the very different circumstances under which a person can donate. The person providing the material may be living or deceased, the material may be used almost immediately or stored for long periods of time, or the material may be used unprocessed or heavily processed. Whatever the case, handling and disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and for the human body.

Ethical standards of all aspects of tissue and cell donation and transplantation have to conform to the *Oviedo Convention on human rights and biomedicine* (1997) [17], and the *Additional Protocol on transplantation of organs and tissues of human origin* (2002) [18]. In addition, all EU member states must comply with European Directive 2004/23/EC *on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of*

human tissues and cells [39]. Other important guidelines to observe from an ethical viewpoint are *Committee of Ministers Resolution (1978) 29 on harmonisation of legislation of member States relating to removal, grafting and transplantation of human substances* [23], *WHO Guiding Principles on human cell, tissue and organ transplantation* [31] and the *Declaration of Istanbul on organ trafficking and transplant tourism* [33].

Any action in the field of tissues and cells for human application should be carried out in accordance with professional obligations and standard procedures.

A very wide range of tissues and cells may be donated. Many of these tissues and cells may be donated only after death, but some may be provided by living donors. Tissue donated for transplantation after death is governed by the same ethical principles as organs; it enters a common pool to be used according to need and its use cannot be directed to a particular individual. Cells such as those from the bone marrow can be donated by a living person and directed for transplantation to another specific person. Similarly, gametes may be donated for use within the couple but may also be donated for altruistic reasons. For tissue donation from deceased individuals, the 'dead-donor rule' (which states that patients must be declared dead before removal of any vital organs or tissues for transplantation) must be strictly respected [62].

1.9.1. Consent

The Oviedo Convention states that an intervention in the health field may be carried out only after the person concerned has given free and informed consent to it. This person must make a free choice in the absence of any undue influence and be given appropriate information beforehand as to the intended use and nature of the intervention as well as its consequences and risks. The person concerned may freely withdraw consent at any time.

The *Additional Protocol to the Convention on human rights and biomedicine concerning transplantation of organs and tissues of human*

origin expands these provisions further for the specific case of donation and transplantation. These provisions are explained further in detail in Chapter 4. Specific cases related to consent in ART procedures are outlined in Chapter 25.

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, explicit and scrupulously respected.

The recipient and, if adequate, the person or official body providing authorisation for the human application, must be given appropriate information beforehand as to the purpose and nature of the procedure, its consequences and risks, as well as on 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 through obtaining consent, the risk of nefarious trading and potential physical harm from the use of transplantable tissue for human application will be reduced.

1.9.2. Conflicts of interest

To avoid any potential conflict of interests, physicians determining the death of a potential donor should not be directly involved in the tissue or cell procurement from the donor or subsequent transplantation procedures, 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 are given realistic estimations of the prospects of success of their treatments based on their age and specific medical circumstances. Similarly, the welfare of potential donors (especially with respect to 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.

1.9.3. Financial aspects of donation and transplantation

Discussions around how best to increase supply of human tissues and cells often focus on questions of donor motivation, i.e. 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. This motion 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 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 case 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 donate must not render a decision to donate non-altruistic. Initiatives that reduce the barriers to donation should only facilitate individuals to carry 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 interventions, both of which aim at increasing donation by changing its costs and benefits [63]. 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 promulgated 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. Com-

pensation to donors should cover only reasonable expenses and should not act as an incentive or inducement (both direct and indirect).

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 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. Tissues and cells should be supplied on a procurement cost basis and no payment should ever exceed the justifiable fee for the services rendered. Professional bodies should ensure that their guidelines reflect their members' responsibilities in the acquisition and supply of human tissue. Tissue establishments should operate as professional organisations on a non-profit-making basis and not as commercial organisations.

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

1.9.4. Equitable access to transplantation or to assisted reproductive technology treatment

Healthcare in general is a human right because it secures and protects access of people to the normal range of opportunities and because

it allows people to thrive. Given the importance of health for the general wellbeing of a person, every person, regardless of his/her income or financial means, should have access to a decent minimum of healthcare.

Requests or the demand for human tissues and cells in many instances exceed what is available or the supply. Significant practical and ethical questions regarding efficiency and fairness arise as to how to distribute these limited resources. Article 3 of the *Additional Protocol to the Convention on human rights and biomedicine concerning transplantation of organs and tissues of human origin* states that transplantation systems must exist to provide equity in access to transplantation services for patients. Except in the case of direct donations, tissues and cells must be allocated among patients only in conformity with transparent, objective and duly justified rules according to medical criteria. The persons or official bodies responsible for the allocation decision must be designated within this framework.

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

Inclusion of infertility treatment in the basic healthcare tier is dependent upon the general level of welfare in society. Cultural, educational and religious backgrounds may also affect the availability of these therapies. Nevertheless, given the rightful claims of other types of healthcare and other fundamental needs in society (as well a limited availability of non-partner gametes and embryos), access

cannot be expected to be unlimited. Ultimately, access to ART should be considered in a structured way to include efficiency, safety and equity to avoid discrimination [64]. Some countries have opted to give public access to a fixed number of cycles/treatments for everyone even if this means that those who need more treatment have to pay for it themselves.

Cross-border reproductive care refers to a widespread phenomenon whereby patients seeking ART treatment cross international borders to obtain reproductive treatment outside their country of residence. The reasons for travelling vary between countries, but the most common reason is law evasion if the technique is forbidden *per se* or if a particular group is excluded from treatment. There may be other limitations to access at home (e.g. long waiting lists). Other reasons for travelling are better quality of care and less expensive treatment [65, 66].

The ideal situation is fair access to fertility treatment at home for all patients. This ideal should be promoted at all levels [66]. However, if for some reason treatment at home is not possible or not available, cross-border reproductive care may provide a solution for patients. Furthermore, it is in accordance with the principle of freedom of movement of patients within Europe [67]. However, crossing borders may also lead to increased shortage of scarce resources in the visited country and to the detriment of local citizens. Health Authorities may want to introduce a system for fair allocation of scarce resources that takes into account local needs, such as a maximum number (or percentage) of treatments provided to foreign patients.

1.9.5. Equity in donation

Individual motivation and choice is only one part of the donation picture; the central role of organisations, organisational procedures and professionals in facilitating donation should not be underestimated, or indeed the importance of trust in these systems. An example of such organisational aspects is that, whenever a person

dies in circumstances where donation is a possibility, this possibility should be raised with their family.

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

As discussed above, introduction of financial incentives for donation renders certain social groups (and especially women because they are oocyte donors) in the field of ART particularly susceptible to disparities based on social and economic status.

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

1.9.6. Anonymity

The identity of the donor and recipient should (except in the case of donation between persons having a close personal relationship) be maintained in strict confidentiality. Such precautions will prevent abuse and protect the families of donors and recipients from feelings

of anxiety associated with emotional involvement, obligation to return favours, or guilt.

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

1.9.7. Transparency

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

Transparency can be achieved by maintaining public access to regularly updated comprehensive data on processes; in particular allocation, transplant activities and outcomes for both recipients and living donors, as well as data on organisation, budgets and funding. Such transparency is not inconsistent with shielding from public-access information that could identify individual donors or recipients, whilst still respecting the requirement of traceability. The objective of the system should be not only to maximise the availability of data for scholarly study and governmental oversight but also to identify

risks (and facilitate their mitigation) to minimise harm to donors and recipients.

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Chapter 2

Quality management, risk management and validation

2.1. Introduction

This chapter outlines the general principles of quality management systems (QMS) that should be applied in 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 three 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 carried out.

b. The quality management system, 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.

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 provides tools to support a tissue establishment in the construction of a robust and efficient programme:

- The International Organization for Standardization (ISO) requirements, as addressed in the ISO 9000 Quality Management System 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 European Union (EU)-funded project EuroGTP, which aimed to agree harmonised practices and techniques across Europe and to increase the know-how and the level of competence of tissue establishment personnel. Much of the guidance developed in this project has been incorporated in the chapters of this Guide.
- 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 the tissue establishment QMS; these requirements are legally binding in EU member states.
- Foundation for the Accreditation of Cellular Therapy (FACT)-Joint Accreditation Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation (JACIE) International Stand-

ards for cellular therapy product collection, processing and administration.

- 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 quality management 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-quality system addresses quality management under the following headings:

a. Personnel and organisation;

b. Premises, equipment and materials;

c. Contractual arrangements;

d. Documentation and record keeping;

e. Quality control;

f. Quarantine and release;

g. Process validation;

h. Traceability;

i. Complaints;

j. Investigation and reporting of non-conformance, adverse events and reactions;

k. Recall;

l. Self-assessment, internal and external audit;

m. Quality risk management;

n. Fiscal and continuity planning;

o. 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 hierarchical 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 documented delegate 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. Tissue and cell manipulation 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. Training methods must be documented and training records maintained. The effectiveness of training programmes should be monitored by regular assessment of the competency 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 the routine or occasional nature of the activity.

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. Standard universal precautions 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, with prophylaxis for the transmissible agent(s) where appropriate.

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, equipment and materials

Premises and equipment 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.

2.4.1. Premises

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 qualified air systems that make them suitable for the procurement of tissues that are not subsequently sterilised. Other types of facilities, 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 Chapter 6 and in Part B of this Guide.

Premises should include adequate dedicated areas that allow the 'first in, 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 quality/safety of the tissues and cells directly (e.g. an additive) or indirectly (e.g. donor testing kits). These areas should allow for adequate segregation of those materials in quarantine from those released for use. They should be temperature-mapped and monitored where appropriate.

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 (SOP) 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 7 and in Part B of this Guide.

Processing facilities should be dedicated to this activity and should be designed, qualified 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 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). Many 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 7 and in Part B of this Guide.

2.4.2. Equipment

A list or register of equipment that might influence the quality or safety of the tissues or cells should be maintained. All equipment on this list must be designed, qualified and maintained to suit its intended purpose and minimise any hazard to donors, recipients, operators or to the quality and safety of the tissues and cells. A validation plan should indicate when and how critical pieces of equipment should be qualified and re-qualified as necessary (see section 2.9).

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.

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.

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 a SOP that provides specific requirements for the calibration of each measuring device such as defining the frequency of calibration, number of measurement repeats, 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 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.

2.4.3. Materials

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.

Inventory records must be kept for traceability and to prevent use of materials after their expiry date. Each batch of critical reagents or consumables must be traceable to the respective procurement or processing session of tissues and cells in which they were used.

Apparent deviations in the quality and/or performance of equipment and materials must be investigated and documented promptly. Outcomes of these investigations should be reported in a timely manner to the RP, who should consider and approve the corrective and preventive actions to be implemented. For relevant deviations, a notice should be sent to the manufacturer and, where appropriate, reported to the Health Authority.

Further guidance on reagents and materials used in tissue and cell processing is provided in Chapter 7.

2.5. Contractual arrangements

Where steps influencing the quality or safety of tissues or cells (i.e. critical steps) are carried out by a third party, there must be a contract or Service Level Agreement in place that describes the roles and responsibilities of all parties for maintaining the quality chain and the quality requirements for the service provided. Agreements should allow for on-site audits of contracted third parties to confirm their compliance to expectations. An example of an expectation is that if a supplier changes specifications for equipment or reagents they provide to a tissue establishment, or if they substitute an item for the usual one ordered, they must first be certain that these changes are acceptable to the tissue establishment.

In EU member states, tissue establishments must establish written agreements with a third party each time an external activity takes place that influences the quality and safety of tissues and cells pro-

cessed in co-operation with a third party. They must keep a complete list of these agreements and make them available at the request of Competent Authorities.

Agreements must be dated, reviewed and renewed on a regular basis.

Written agreements should be in place for at least the following service suppliers:

a. testing laboratories (including donor, tissue and environmental testing);

b. procurement teams that are independent from a tissue establishment;

c. transport companies;

d. suppliers of critical equipment, consumables and reagents;

e. suppliers of services such as tissue and cell storage, processing or sterilisation;

f. end users of tissues and cells.

Once tissues and cells have been distributed for clinical application, they usually leave the QMS of the tissue or cell facility. However, appropriate control of transport and storage conditions, appropriate handling and preparation before use, and full traceability must be maintained. Maintenance of quality and traceability is usually achieved through providing users with clear and detailed written instructions. It is advisable to also ask clinical-user facilities to sign a simple agreement taking responsibility for continuation of the quality chain. This is essential, for example, in the case of a sperm bank providing sperm to an ART clinic for embryo creation and clinical application. However, it is not necessary for partner donation treatment in an ART centre or autologous bone-marrow transplantation where all steps (including clinical application) are carried out in the same facility.

Distribution direct to the patient for use without supervision of a health professional (e.g. autologous serum drops) requires particular attention to instructions for storage and use, and should be carried out

only if it is the only available option. Direct distribution of sperm to individuals for use without supervision of a health professional should be avoided.

2.6. Documentation and record-keeping

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. 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 SOP 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 on 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 SOP and forms, must be approved by appropriate and authorised persons and be part of a document control system that ensures 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. 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, quality system documentation (including 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. International and national regulations on data protection have to be respected. Personnel should only have access to those categories of data for which they are authorised. See Chapter 11 for further guidance on computerised systems (including requirements for their validation).

Quality specifications should be prepared for each type of tissue and cell graft; these should be the basis for quality control testing and product release.

2.7. Quality control

'Quality control' refers to those activities, such as verification steps, sampling and testing, which are used to ensure that materials, processes and the final product meet the required specifications. Internal quality control in a testing laboratory includes use of positive, weakly positive or negative control samples as appropriate. External quality assessment (sometimes also called 'proficiency testing') involves analysis of unknown samples and evaluation of the results by a third party. Quality control of critical functions can be undertaken using audit techniques that include a sampling plan (see also 7.5.1).

Detailed guidance on microbiological testing is provided in Chapter 8. Guidance on specific quality-control tests for specific types of tissues and cells is provided in Part B of this Guide.

2.8. Quarantine and release

All tissues and cells must be stored with an unambiguous quarantine status until all quality-control tests and checks have been conducted and the results reviewed by the individual responsible for release. Release of tissues and cells may be conducted in two steps. The first step confirms compliance of the donor with defined acceptance criteria (which is usually carried out by clinical personnel). The second step confirms compliance of the tissues or cells themselves, their characteristics, processing and storage, with those criteria defined in the product specification. The latter is usually carried out by quality assurance personnel. The concept of 'quarantine and release' is not applicable to partner donation in ART and to some types of autologous or direct donation (see section 10.5 for guidance on Exceptional Release). Tissues and cells that cannot be categorised as 'released' during storage must be stored with an unambiguous quarantine status.

2.9. Validation and qualification

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

2.9.1. Qualification of facilities and equipment

Facilities and equipment should be qualified following the four steps shown below.

2.9.1.1. *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).

2.9.1.2. *Installation qualification*

Installation qualification (IQ) should be carried out on new or modified facilities, systems and equipment. 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;

d. verification of construction materials.

2.9.1.3. *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 preventative mainte-

nance requirements to be finalised. It should permit a formal 'release' of the facilities, systems and equipment.

2.9.1.4. *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 statistical significance in relation to the volume of activity at the tissue establishment. Although PQ is described as a separate activity, in some cases it may be appropriate to undertake it in conjunction with OQ.

2.9.2. General principles of process validation

Processes applied to tissues and cells should be validated to demonstrate that they do not have detrimental effects on the required characteristics of the product, and that they meet any claims on the label (e.g. a claim of sterilisation). A risk-assessment approach should be used to determine the scope and extent of validation, which should be proportionate to the degree of risk. Process validation should normally be completed before distribution and clinical use of new components ('prospective validation'), or during routine processes ('concurrent validation'), or for processes that have been in use for some time ('retrospective validation'). Any change to a process should be assessed for impact on the status of the validation of that process.

Process validation studies must be conducted on typical processing scale batches. The number of batches used will depend on the variability of the process, the complexity of the process, the variability in the required characteristics of the particular tissues and cells and the experience of the technicians, but will usually include at least three consecutive batches.

2.9.3. Planning for validation

The first steps involve identification of the requirements for the procedure or process and documentation of this specification (e.g. in a validation master plan [VMP]). A VMP should be a summary document that should contain data on at least 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.

2.9.4. Validation documentation

A written protocol should be established that specifies how validation should be conducted. The protocol should be reviewed and approved, and specify the critical steps and acceptance criteria. Any changes of the plan should be documented with appropriate justifications.

2.9.5. Prospective validation

Prospective validation should include the following:

a. short description of the process;

b. summary of the critical processing steps to be investigated;

c. list of the equipment/facilities to be used;

d. list of analytical methods (as appropriate);

e. proposed in-process control with acceptance criteria;

f. additional testing to be carried out;

g. sampling plan;

h. methods for recording and evaluating results;

i. responsibilities;

j. proposed timetable.

Validation studies should be undertaken by applying worst-case scenarios. The equipment used for validation studies should be fully qualified, and measuring devices should be calibrated to traceable standards.

Detailed guidance on process validation is provided in Chapter 7.

2.10. Change control

Change control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a product of the desired quality, and which is consistent with the approved specifications. Written procedures should be in place to describe the actions to be taken if a change is proposed to a starting material, final product specification, equipment, environment (or site), method of production or testing, any other change that may affect the quality of tissues or cells or the reproducibility of the process. All such changes should be requested, documented and accepted formally. The likely impact of the change of facilities, systems and equipment on the final product should be evaluated (including a risk analysis). The need for, and the extent of, re-qualification and re-validation should be determined.

The training programme should be re-assessed for any critical change in environment, equipment or processes. Training records (including plans and training protocols) must ensure that training needs are 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 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 13.

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. The latter should then be investigated thoroughly, including root-cause analysis and identification of corrective measures (see section 2.13 for details).

2.13. Investigation and reporting of non-conformance, adverse events and adverse reactions

Examples of non-conformance include deviations from SOP, errors and accidents. Non-conformance 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 non-conformance and a log of all the instances of non-conformance that are investigated, including detailed documentation of the investigation, root-cause analysis and corrective/preventive actions taken. A categorisation of cases of non-conformance depending on criticality with respect 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 non-conformance should be carried out to identify common failures and identify areas for concern.

Serious adverse events and serious adverse events reactions should be reported through a vigilance system. For detailed guidance on vigilance of tissues and cells, see Chapter 14. If products containing tissues or cells are classified as in the EU ATMPs, adverse occurrences should be reported through pharmacovigilance systems for process events and 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 as not meeting required quality or safety requirements. This written procedure must encompass the need to agree and document any corrective and preventive actions that may be necessary. The actions should be communicated to the end user, where appropriate. Further guidance on recall is provided in Chapter 14.

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. Quality risk management

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

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

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

Risk assessment should be repeated and documented whenever a critical process is changed.

Risk assessment is also an essential tool for making important decisions, particularly when departures from normal procedures are under consideration. Examples would include:

a. selection of a donor where full compliance with the normal criteria are not met, but the donation has a particular clinical value

and the risk can be mitigated sufficiently to justify the departure from normal procedures;

b. exceptional release of non-complying tissues or cells on the basis that the potential benefits for the recipient and the lack of availability of alternatives outweigh the risks;

c. retention or discard of tissues and cells in storage following the introduction of new procedures or tests that imply higher levels of safety or quality;

d. eligibility determination where certain test results are reactive, for example, EU Directive 2006/17/EC Annex 2 requires further investigations with a risk assessment when antibody to hepatitis B core antigen (anti-HBc) is positive and hepatitis B surface antigen (HBsAg) is negative and where a donor is reactive for a Treponema-specific test (see chapter 5 for further guidance on donor testing);

e. prioritisation of a list of corrective actions following an audit or inspection, or prioritisation of quality improvements in general.

The approach to risk assessment should be systematic and should be documented. The most commonly applied risk assessment methods are Failure Mode and Effects Analysis (FMEA), Failure Mode, Effects and Criticality Analysis (FMECA) and Hazard Analysis and Critical Control Points (HACCP). These methods estimate severity and probability, but FMECA also includes a factor for detectability, taking into consideration that those hazards that are more easily detected represent a lower overall risk. FMECA allows the estimation of a risk priority number (RPN) for the ranking of identified risks. The estimated level of risk should be considered in the light of benefits, of alternatives, and of the costs associated with reducing risk further. An organisation should have a clear policy regarding risk acceptance.

Undertaking risk assessment at various stages helps to define requirements and alternatives, aids in the process of supplier selection, and helps to determine the scope and extent of validation. The method-

ology has been used for tissue and cell banking for specific process steps [3] and for reviews of an entire process [4].

Risk assessment is not a 'one-off' process but a cyclical one (Figure 2.1). Risk assessment should be followed by risk avoidance and reduction (if possible) and continuous re-evaluation of residual risk.

Figure 2.1. Cycle of risk assessment

Guidance on quality risk management is provided in Part III Q9 of the Rules governing medicinal products in the EU, Volume 4: *EU Guidelines for good manufacturing practice for medicinal products for human and veterinary use* [1], in which several well-established risk-assessment methodologies are listed. Inclusion of this new section in GMP guidance reflects the current thinking that risk management should be an integral part of quality management.

2.17. 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 (Service Level Agreement or contract) with another organisation for the transfer of tissues or cells, documentation and services in these circumstances.

2.18. References

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Chapter 3

Recruitment of potential donors, identification and consent

3.1. Introduction

The evolution and success of implantation/transplantation medicine in recent years has increased the need for tissues and cells. However, implantation/transplantation would not be possible if tissues and cells were not donated.

Autologous donors give their tissues and/or cells to be used for their own treatment. Allogeneic donors donate their tissues and/or cells to another person. The latter type can be living donors or deceased donors.

Living donors can provide gametes, cells that can regenerate (e.g. haematopoietic stem cells [HSC]) and surgical residues (e.g. bone when a femoral head is removed during hip replacement surgery or heart valves from their own heart when they undergo a heart transplant). Living donors can be related or unrelated to the recipients and, in the case of reproductive cells to be used in assisted reproduction technologies (ART), the donor could be a partner donor or a non-partner donor. For ART, this chapter only applies to non-partner donation.

In this chapter we describe the requirements related to the recruitment, identification and consent for living and deceased donors.

The *Convention on human rights and biomedicine* sets out the requirements for consent for any medical intervention, including the removal of tissues and cells from living and deceased donors for the purpose of transplantation into a recipient. All those who procure, process, store and transplant donated tissues and cells must assure themselves that consent, in accordance with domestic law, has been obtained for the donation. Tissues and cells must be used only for the purpose for which consent has been given.

The *Additional Protocol to the Oviedo Convention concerning transplantation of organs and tissues of human origin* also prohibits trafficking of organs and tissues. Prohibition of financial gain from the human body or its parts requires that consent must be voluntary, without any incentive or inducement to donate or consent to the donation. It follows that donation must not create any obligation on the part of the donor, apart from those obligations associated with ensuring the safety and traceability of the donation, or to the recipient. This prohibition of financial gain does not prevent compensation for loss of earnings during the donation procedure and reimbursement for legitimate medical and technical services supporting the removal, processing and storage of tissues and cells for transplantation.

Before tissues and cells are procured, it should be documented that consent for the procurement has been obtained in accordance with national legislation, and that the donor was identified correctly and by whom. The information for the donor should specify whether the samples will be obtained for therapeutic or research purposes, or both.

3.2. Living donors

3.2.1. Donor recruitment

Donor recruitment, especially for the donation of bone marrow and HSC, is a continuous process. It includes increasing public awareness and educating the public at local and national levels about the

benefits of transplantation. Recruitment drives focus on altruism, solidarity and social engagement, and are aimed at people who are likely to come forward and provide their details to a registry. Personal stories by the family members and friends of patients often result in increased media interest and can be used to communicate the importance of registration and donation to any patient that could need it. Once registered, the potential donors can be contacted if and when a patient needs a donation. It is vital that the recruitment and selection procedures for donors are sufficiently robust to ensure the safety of the donor and quality of the donated tissues and cells.

Some registries recruit donors directly, whereas other registries have agreements with organisations which recruit donors and list the donors on the registry. Blood banks and transfusion centres have an active role in helping to recruit donors.

The registry or donor centre has an obligation to respect the donor's rights and to act in an ethical manner. It must be made clear that donation is voluntary and that donors have the right to withdraw their consent to donate at any time. Registries and donor centres should feel responsible for the care and wellbeing of the donor, and be aware of the appropriate standards to ensure donor safety during and after the donation process.

A registry or donor centre should provide potential adult donors with appropriate information with a view to enable the person concerned to give written informed consent. The registry or donor centre is also responsible for coordinating the testing of prospective volunteer donors. At the time of registration, donors should be made aware that they must be physically able to donate and, in the case of HSC, donors must be made aware that they may be asked to donate bone marrow or stem cells contained within peripheral blood. Children should become donors only in very specific circumstances and never through public registries.

Potential donors must complete forms giving their personal details and provide blood/saliva for DNA and human leucocyte antigen (HLA) typing. The registrants' information (which includes relevant

personal data and information on HLA typing) must be stored in a registry. Registries can also list donors on international databases, and are responsible for communicating with transplant centres. The donor's written informed consent must be obtained for typing and storing his/her personal data in the database of the registry or donor centre.

The matching process must be explained to the donor. It should be explained that registering as a donor does not mean he/she will be asked automatically to donate stem cells or bone marrow. Donor registries should take all possible steps to monitor and review the retention and availability of donors.

If a donor is selected to donate, the type of collection process required should be discussed with the donor during the work-up process by taking into account the needs of the patient. The donor's decision regarding this process must be respected. If the donor does not agree to undergo the collection procedure requested by the transplant centre, it may be necessary to look for an alternative donor.

In the case of reproductive cells, recruitment of potential non-partner donors should be based on the principles of voluntary, informed and non-profit donation. Publicising and/or advertising the need or the availability of donated gametes should be discouraged (in some European Union (EU) countries such behaviour is forbidden by domestic laws). Oocyte sharing is allowed in some European countries.

National registries for gamete donation should be in place to keep track of the number of donations per donor, number of children born after donation, and serious adverse events or adverse reactions relating to the donations. Nationally accepted limits of the maximum number of donations and/or children born are strongly recommended.

Upon donation of any tissue or cell, it is crucial to guarantee that removal of financial barriers to donate must not render a decision to donate non-altruistic. Financial incentives to donate are particularly worrisome in the case of gamete donors (especially oocyte donors) because they may change the donor's perception of the relative risks and benefits of a donation, which is not free of potential health

hazards and psychological consequences. For more details on the financial aspects of donation, please refer to Chapter 1.

3.2.2. Consent/authorisation of donors

A person may donate tissues and cells only after giving free and informed consent to doing so. This person must beforehand be given appropriate information as to the purpose and nature of the intervention, as well as on its consequences and risks. The scope and duration of the consent must be stated explicitly.

If, according to the law, a person does not have the capacity to consent to donation, donation may be carried out only with the authorisation of his/her representative or an authority, person or body provided by law. The Oviedo Convention provides protection for those who do not have the capacity to give consent, such as minors or legally incompetent persons. A general principle under these circumstances is that the person must, as far as possible, take part in the authorisation procedure. In the case of a minor, his/her opinion should be taken into consideration in proportion to his/her age and level of maturity. The recipient could be a brother or sister of the donor or, in some cases a person, who has a very close relationship with the donor. There are also specific instances, defined in domestic law, in which donation can take place if authorisation is given in writing by a person or body specified by law, and if the authorisation process has been approved by a Health Authority.

The donor must be informed of the types of tissues and cells to be removed, any associated risk to the donor, the purpose or use to which the tissues and cells will be put, whether or not the tissues and cells are to be processed and stored. The discussion on consent should be conducted in a suitable environment. The donor must be informed of the transmissible infections that might pose a risk to the recipient, and what would happen in the event of a positive result. Donors must also be informed of after-care, follow-up and potential adverse reactions and events due to the donation procedure. The donor should be made aware of the procedures in place to protect donor data.

The person who requests the consent of the donor should have received specific training for this purpose, must be sensitive to the needs of the donor, and must be able to answer questions about donation and transplantation processes.

It is advisable to seek written consent. If verbal consent is obtained, it must be documented clearly. Documented consent should be made available to the tissue establishments that receive the material for processing and storage to confirm that consent has been duly given.

For donors donating HSC, consent must be obtained at several stages: matching, testing, and donation. It is recommended that written consent be obtained at each stage.

All results from donor evaluation should be made available to the donor. If a donor is found not to be suitable, the results of donor assessment should be discussed with the donor, and they should be advised if they need to take any action regarding their health. If the findings do not exclude the donor but may have implications for the recipient (e.g. HSC donor who is a carrier for haemoglobinopathy) then the transplant centre should also be informed.

The donor should also be informed of his/her right to withdraw from the procedure and the consequences for the recipient if the donor removes consent in the middle of the procedure. These consequences may be particularly severe (e.g. if a HSC recipient has already begun a preparatory regimen to receive a HSC transplant).

3.3. The deceased donor

Tissues such as bone, soft tissue (fascia, tendons, ligaments), skin, cornea, heart valves and/or vessels and specific cell types from certain tissues (e.g. mesenchymal stem cells from bone, skin or adipose tissue) can be removed from deceased persons and used for patient treatment. Deceased donors must be treated with respect. Every effort should be made to restore the appearance of the body after removal of any tissue.

3.3.1. Donor detection

In many cases, donors register their intention to donate tissues after their death. However, systems should be in place to identify potential donors among deceased persons who have not registered their intention to donate tissues. Detection of such donors can increase the availability of tissue allografts for patients who need them, provided the donation can proceed in accordance with domestic law. Similar systems can be used to detect potential tissue donors and organ donors.

Donors should be screened using medical records, interviews with medical staff that treated the donor (attending physician, general practitioner, nurse) and from relevant information provided by the donor's relatives or other persons who have information on the donor's social and medical history. Key selection criteria must be taken into consideration before a donor is accepted. These criteria vary depending on the type of tissue to be retrieved and implanted into a recipient (see Chapter 4). Risk factors such as the potential donor's sexual behaviour, travel and exposure to sources of infection must be evaluated.

In the event that a health facility does not have the means to manage a potential tissue donor, or is not licensed to procure bodily material according to domestic regulations, a local network should be in place that arranges the transfer of potential donors to a hospital facility or procurement centre.

3.3.2. Consent/authorisation for deceased donation

Countries use the explicit ('opt-in') system of consent, or the presumed ('opt-out') system of consent for removal of tissues from deceased donors for transplantation.

For presumed consent for tissue donation to be ethically acceptable, it must be clear that the population is adequately informed about the procedure and implications of donation, in just the same way as an individual would be informed if providing explicit consent.

The system of presumed consent has been credited with helping to increase the number of donations. However, other measures (e.g.

an effective system to identify potential donors and more effective management of deceased donors) have also resulted in increasing the availability of tissues from deceased donors.

3.3.2.1. *Explicit or opt-in system of consent*

The opt-in system requires each individual or, his/her relatives, once the person has died, to make a conscious choice to donate tissues and cells. Consent for donation must be given by an appropriate person in accordance with domestic law. The discussion on consent must cover the scope and duration of consent, and the fact that consent can be withdrawn.

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, Wales 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, longstanding friend) and states that consent should be obtained from the person ranked highest in the hierarchy.

An individual can make their wish to be a tissue donor known during their lifetime by joining their national organ/tissue donor registry. In some countries, those who apply for a passport or driving licence also have to state whether or not they are willing to donate tissues and cells after death. Some countries have donor cards which document the decision. These documents can then be used to determine whether or not the person wished to be a tissue donor. A person can also express their wish not to be a donor.

3.3.2.2. *Presumed, deemed or opt-out system of consent*

In this system, a person is presumed to have given consent to donate tissues and cells if he/she has not stated his/her wish to opt out of

becoming a donor. Most people support donation of organs and tissues but history shows that only a limited number will actively sign up to donate tissues and organs if given the opportunity. Hence, several countries have passed laws which assume that consent is in place unless the person opted out or decided that they did not want to donate their tissues after death.

Several countries have presumed-consent policies, including Spain, Italy, France, Belgium, Austria and Singapore. These opt-out systems were introduced to help meet the shortfall in organs, tissues and cells available for transplantation. However, in most programmes, if it emerges that the family is against the idea of the donation proceeding, tissue procurement will not proceed.

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.

3.4. Data protection and confidentiality

Confidentiality and protection of all information regarding the donor and recipient is considered of utmost importance to ensure public confidence in the procedures used for procurement and transplantation. Domestic regulations should be in place to ensure that all information on each donation event is confidential.

Security measures should be in place for data protection of the identity of the donor as well as the recipient. Such measures should ensure that unauthorised access to information and unauthorised changes to stored data or donor files do not take place. Standard operating procedures should be in place to resolve any discrepancies in stored data.

A system for the assignment of unique codes to the donor, donation procedure and donated material, as well as the recipients, which can be linked in a traceability system, should be in place (see Chapter 13).

In the EU, Directive 95/46/EC on data protection applies to personal data processed throughout all activities from donation to transplantation of tissues and cells.

Related document:
Appendix 4 – Sample consent form

Chapter 4

Donor evaluation

4.1. Introduction

Evaluation of any candidate for the donation of tissues and cells includes comprehensive collection of clinical and personal information to determine the risks associated with various transmissible diseases (e.g. viral, bacterial, mycobacterial, fungal, parasitic, prion-associated, malignancies), as well as obtaining the donor's medical history to assist in assessment of the quality of the tissues and cells that may be procured. This critical step of screening a potential donor is one of the most important factors in providing safe cells or tissues for human application or assisted reproduction, and no test or analyses can replace it. Standard operating procedures must be in place for acquiring all the data necessary to make an informed decision regarding the suitability of each donation.

In general, donor evaluation consists of:

a. collecting information about the donor's health that identifies generic and specific contraindications for donation of tissues and cells. It is necessary to collect information regarding:
   i. medical history (including a family history of genetic disease),
   ii. social and behavioural information (including travel history);

b. physical examination;

c. serological and microbiological testing and other additional complementary tests (detailed in Chapters 5 and 8);

d. additional information (e.g. autopsy report) that provides extra information from internal examination in the case of deceased donors.

During donor evaluation, confirmation of the consent and appropriate confirmation of donor identity (see Chapter 3) are also essential steps.

For European Union (EU) member states, the selection criteria for deceased donors (including additional exclusion criteria for deceased children donors) and living donors of tissues and cells are specified in Annex I of Directive 2006/17EC.

4.2. Donor information

A risk-assessment interview for the donor is a critical medical record and must be developed and referenced in the policy and procedures manual of the tissue establishment. It is undertaken using a questionnaire and must cover all relevant risks associated with medical history, as well as history of transmissible diseases, behaviour (lifestyle, travel) and, if relevant, of the family. The interview should be done, documented and signed by a trained and competent authorised person. It should be held in private and should be carried out ideally just before donation or as soon as possible after procurement in certain circumstances, such as donation of surgical residues or cord blood or if the ischaemia time is too short to allow it to be conducted beforehand.

The risk-assessment interview must be carried out directly with living donors or, in the case of a deceased donor, with a knowledgeable person(s) (treating physician, partner, family, or a person who knew the donor well) who can give personal information to help determine eligibility for donation. No release of tissues from a donor should ever proceed without this interview. When interviewing a person representing a deceased donor (partner, family) care must be taken during this interview to consider their emotional state. However, this should

not override the need to properly screen the donor for relevant risks. Data from such interviews can reveal risk information not found in available medical records. To obtain complete and honest answers (and to avoid misunderstandings about the objective of certain questions of a private nature), a clear explanation that they are needed to ensure the quality and safety of the tissues and cells should be provided. Interviews can be conducted as a conversation between the interviewer and interviewee to facilitate this objective. Interviewees should be informed that they can ask questions when they do not understand the information being sought or if they feel that there is an issue that the interviewer has not asked about. The interviewee should know the donor well enough to be considered as an acceptable representative and be instructed to answer the questions to the best of his/her knowledge. The interviewee should not simply be the most readily available person. Although a family member may seem appropriate, another individual may be able to provide better, current information. Thus, the donor's sexual partner, girlfriend/boyfriend, current housemate, care-giver or a close friend should also be considered as potential interviewees. It is important to assess whether the selected interviewee has known the donor for a recent period of significant length. Selecting an interviewee that currently lives with the candidate for donation may be preferable because he/she may be able to offer the best, current, behavioural risk information. The interviewer must be capable of deciding whether the interviewee is sufficiently knowledgeable or if another person should be sought. The person eligible to give consent may not be the most informed person to be interviewed for evaluation of behavioural or medical risks. The interview should be conducted using terminology that can be easily understood by the general public. Interviewers should avoid using medical terms when possible and short, direct questions are the best way to ensure that the questions are understood.

All information should be secured on a donor evaluation form or in a donor evaluation application. An example of a donor risk-assessment questionnaire (together with a framework document for its rationale,

both provided by National Health Service Blood and Transplant, UK) is available in Appendix 5.

All available information obtained during initial screening should be taken into account before donor clearance for procurement and banking.

There are special considerations for evaluating a paediatric donor (see section 4.6).

4.2.1. Evaluation of medical history (generic- and tissue-specific contraindications)

The medical history of a donor includes specific information regarding:

a. current clinical information;

b. medication;

c. transfusions/infusions;

d. medical history (diseases, surgeries);

e. family history of genetic disease;

f. cause of death ([COD]; for deceased donors).

Review of the information mentioned above should allow assessment of generic and specific tissue-related contraindications for donation.

4.2.1.1. *Current clinical information*

The attending physician or medical staff caring for the potential donor shall need to answer questions regarding the clinical history and, in the case of a deceased donor, the events leading to death.

If a potential donor appears eligible after initial screening, available records should be obtained, reviewed and evaluated as soon as possible. The types and extent of records that are available can vary, depending whether it is:

a. a living donor (taking into account that some special issues have different implications if it is allogeneic or autologous donation);

b. a deceased donor (whether death occurred within or outside a healthcare facility).

The following records may have been produced and, if so, should be obtained and reviewed:

a. emergency room and emergency medical transport (ambulance) records, if applicable;

b. admission records, progress notes, physician's orders/notes and nursing observations;

c. results of laboratory tests (microbiology, chemistry, haematology, virology, urinalysis, toxicology, genetic screening, pathology);

d. information relating to transfusions and infusions (to be used for evaluation of haemodilution);

e. radiographs, other modes of imaging;

f. surgical records (review for additional transfusions and infusions that may have taken place and any biopsy reports);

g. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling);

h. discharge summary or death record (assess whether an autopsy is planned).

Direct communication with the attending physician or the medical staff caring for the potential donor is recommended because it often provides valuable information. Specific inquiries should be made regarding any concern for the potential donor having an active infection or a communicable disease. Extensive hospitalisation in the donor's medical history can increase the risk of the donor having acquired a nosocomial infection (as seen in potential donors who have undergone prolonged ventilator-assisted breathing). Abnormal, unexpected or unusual results from medical tests undertaken during hospital admission should be scrutinised, such as those from microbiological cultures, biopsy results or haematological evaluations (e.g. very low, abnormally high or a sudden substantial drop in the white blood cell count, or a cell differential evaluation [which describes an

unusual alteration in the morphology of white blood cells or red blood cells]). The presence of certain cells and cell counts can indicate active infection. If a series of complete blood counts (CBC) are available, they should be reviewed for suspicious trends and sudden changes. If a review of blood culture results was requested, then the reason for requesting it should be investigated.

However, information can be obtained from outside of a healthcare facility. In these cases, the following records may be available and, if so, they should be located and reviewed:

a. police records (if available);

b. records from the medical examiner or coroner;

c. records from the extended care facility (assisted living facility);

d. records from a funeral home.

Thorough review of all available records is recommended. In some cases records might be known to exist, but are not readily available. Ideally, all records should be viewed before tissue procurement begins but, if this is not possible, they must at least be reviewed by a Responsible Person (RP) before release of tissues and cells for human application.

The donation record must fully and accurately reflect the relevant information gained from reviewing these records and from discussions with medical or other personnel. Transferring information from records to a new document carries the risk of transcription or interpretation errors. These steps must be carried out by well-trained, competent staff from the procurement organisation or tissue establishment.

4.2.1.2. *Medication*

a. treatment with immunosuppressive agents which can weaken the immune system and thereby influence the outcome of serological tests;

b. all other medication; always carry out a risk assessment for each type of tissue and cell (e.g. corticosteroids can have an impact on the quality of bone tissue).

4.2.1.3. *Transfusions/infusions*

If haemodilution is >50%, serology testing on blood samples withdrawn at the time of procurement may not be reliable. Blood samples before haemodilution (if available) must be used for serology testing of the donor or haemodiluted samples may be tested using validated methods for such cases (see Chapter 5 and Appendix 7 for haemodilution algorithms). Potential haemodilution should be considered in donors with massive trauma, intraoperative blood loss, or ruptured abdominal aneurysms.

4.2.1.4. *Medical history*

Thorough collection of the medical history of the potential donor must be carried out. All medical events, diseases and surgical procedures should be evaluated, including assessment of the effect on the quality and safety of the tissues and cells to be recovered, to ascertain if potential donors qualify for donation. This evaluation should include histories related to, for instance:

a. malignancy (see section 4.3.1);

b. autoimmune diseases;

c. neurological disorders or symptoms;

d. genetic diseases;

e. chronically persistent infection: consider the history of bacterial and protozoic diseases that can lead to chronically persistent infections, including tuberculosis, brucellosis, leprosy, melioidosis, Q fever, chlamydiosis, salmonellosis and tularaemia. Specific attention should be paid to tick/arthropod-borne diseases such as borreliosis, bartonellosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis. The risk of transmitting these infectious agents with specific tissues must be assessed, and negative effects for the recipients excluded [7].

4.2.1.5. *Family history or genetic disease (if relevant)*

For instance:

a. if a blood relative of the donor was definitively diagnosed by a physician to have Creutzfeldt–Jakob disease (CJD), then the donor may have a small, genetic predisposition to this disease if the CJD of the relative did not have an iatrogenic cause. Deceased donors having a family history of non-iatrogenic CJD should be excluded;

b. genetic disorders transmissible through donation of gametes or embryos;

c. the risk associated with human T-cell leukemia virus type 1 (HTLV-1) in a donor may be higher if the donor or his/her parents originated from a high prevalence area.

4.2.1.6. *Cause of death (for deceased donors)*

COD is important and can indicate if the deceased donor had, or is suspected of having had, a transmissible disease or a risk of haemodilution; it can point to other concerns regarding tissue quality (including contamination). If the COD is not known, donation cannot be permitted because the death may have been due to a disease that could be transmitted to recipients of tissues and cells. The only exceptions are if:

a. the differential diagnosis includes no risk of transmissible disease and all generic contraindications are ruled out; or

b. an autopsy clarifies the definitive COD after tissue procurement.

4.2.1.7. *Contraindications to donation*

All information related to current clinical status, medical history, medication, infusions and family history facilitate evaluation of the suitability of the donor and of the specific tissues to be donated.

4.2.1.7.1. *Generic contraindications*

The risk-assessment form must include questions that rule out the following generic contraindications:

a. active systemic infection (e.g. sepsis, viraemia, meningitis, endocarditis). 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. Donors with systemic infection, which is not controlled at the time of donation (including bacterial diseases, systemic viral, fungal 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 eye donation (avascular) (see Chapter 15);

b. haematological malignancy (present or history). Other malignancies are not a generic contraindication and are discussed in section 4.3.1;

c. risk of transmission of diseases caused by prions, for example:

   i. patients diagnosed with CJD, variant Creutzfeldt–Jakob disease (vCJD), or having a family history of non-iatrogenic prion risk;

   ii. any suspicion of prion-associated disease (CJD, vCJD), such as rapid progressive dementia;

   iii. a diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has been ruled out by microscopic examination). If dementia has a primary cause (e.g. dementia of vascular origin), donation can be accepted;

   iv. degenerative or demyelinising disease or a disorder of unknown aetiology involving the central nervous system;

   v. transfused in the UK;

   vi. recipients of hormones derived from the human pituitary gland (e.g. growth hormones);

vii. recipients of tissues or cells of the cornea, sclera and dura mater, as well as persons that have undergone undocumented neurosurgery in which the dura mater may have been used;

d. recent history of vaccination with a live attenuated virus;

e. transplantation with organs;

f. transplantation with xenografts (discussed in section 4.3.3);

g. intoxications (e.g. cyanide, lead, mercury, gold);

h. If ingestion or exposure to a toxic substance caused death, the quality of some types of tissues and cell can be affected, and may cause harm to recipients if those tissues or cells are used for human application;

i. if it is known that the potential donor was excluded or deferred from donating blood by a blood collection establishment, and the specific reason for deferral cannot be discovered, the donor may be considered ineligible for tissue donation.

4.2.1.7.2. *Tissue-specific contraindications*

Certain medical conditions can adversely affect specific tissues and cells which, if recovered, processed and made available for human application, may result in unfavourable outcomes for the recipients of tissue and cells. This risk is evaluated on a case-by-case basis and for specific tissue types. Further specifications are pointed out in Part B (the tissue-specific chapters of this Guide).

4.2.2. Evaluation of behavioural risk

Behavioural risk is evaluated to determine donor suitability. It should include an inquiry into histories that could increase the risk of a transmissible disease such as human immunodeficiency virus (HIV) or viral hepatitis [8].

4.2.2.1. *Behavioural risks include:*

- users of non-prescribed injectable (intravenous, intramuscular, subcutaneous) or intranasal drugs;

- if the patient has overdosed on non-prescribed drugs, then the risk of an infectious disease is increased due to the high-risk behaviour associated with use of recreational drugs. However, this risk should be evaluated on a case-by-case basis and can be mitigated by collecting reliable information;
- history of tattoos, body piercing or acupuncture in the last 4 months when it is not known that only sterile instruments or procedures were used;
- sexually-transmitted disease – increased risk if recent occurrence, especially for genital ulcerative disease (syphilis, gonorrhoea, herpes);
- high-risk sexual behaviour in the previous 12 months, including having sex with a male who had sex with another male, in exchange for money or drugs, with a person from a high-risk region for endemic disease (HIV-1 group O, human T-cell lymphotropic virus [HTLV-I]), with a user of non-prescribed intravenous or intranasal drugs, with a recipient of certain human clotting factor concentrates, with someone who has tested positive for HIV, hepatitis B virus (HBV) or hepatitis C virus (HCV) or with a person with clinically active symptoms who has frequent changes of sexual partners;
- incarceration in the past 12 months (prison or a juvenile correctional facility);
- living with someone who has HBV or clinically active, symptomatic HCV;

4.2.2.2. *Exposure events (travel, residency, occupation)*

Exposure events that increase the risk of acquiring a communicable disease can occur at any time during life. This includes accidents, certain medical therapies and travel to, or residency in, an area endemic for certain diseases, for example:

- travelling through, resident or native from a malaria-endemic area;

- a recent bite from an animal suspected of having rabies;
- exposure to someone else's blood when that person was known to be infected with HIV, HBV or HCV;
- sharing a residence with someone who has HBV or clinically-active HCV (in the past 12 months);
- chronically transfused with blood or blood products (concern is raised if the administration of blood or blood products occurred many years ago, before adequate disease screening tests became available);
- occupational or other exposure to a toxic substance in amounts sufficient to affect tissues and cells and affect the outcome of human application (e.g. ethylene glycol);
- new and emerging diseases.

New and emerging diseases (including those that have spread to a new geographical area) can pose a significant challenge when screening donors for risks of communicable disease due to travel history. Professionals responsible for donor selection should be vigilant regarding surveillance of changes to the global movement of infectious disease risks. In Europe, regular monitoring of the Rapid Communication Reports originating from the Eurosurveillance website is recommended, as well as actively seeking information to assess the epidemiological status of diseases in the areas in which a donor has lived or travelled [5]. The risk of transmission of an infectious agent through procurement of tissues or cells from a donor who may have visited an affected area should be balanced by considering the likelihood of a transmission occurring. Regional risks can vary. Some new or emerging diseases that should be considered are: Middle East respiratory syndrome, dengue fever, yellow fever, malaria, Chagas disease, tuberculosis, plague, Chikungunya virus, West Nile virus (WNV), Q fever, antibiotic-resistant diseases, vCJD and HIV-1 group O, rabies, Ebola virus.

In 2012, the European Centre for Disease Prevention and Control (ECDC) in collaboration with the European Commission organ-

ised an expert consultation on priority-setting for risk assessment of communicable diseases transmissible through substances of human origin (blood, tissues and cells, organs) [7]. It was determined that arthropod-borne diseases posed the most urgent threat. West Nile fever, dengue, Chikungunya virus, malaria, Chagas disease and leishmaniasis were identified as 'urgent threats', whereas Usutu virus fever, tickborne encephalitis and babesiosis were not seen as urgent threats. ECDC will use this priority list as a basis for developing comprehensive, review-based risk assessments in 2012, which will be available on the ECDC website [7].

4.3. 'Hot topics'

This Guide aims to be a permanently updated document that allows professionals to be aware and updated on any changes to good practices regarding donation of tissues and cells. Various EU projects have provided technical content which is used here. In the Euro-Good Tissue Practices (Euro-GTPs) project, specific topics that require more extensive work to evaluate if the current criteria of exclusion or acceptance should be changed, or topics where there is not yet consensus on how to evaluate risk, have been classified as 'hot topics'.

These hot topics will change from one edition of this Guide to the next. Some will be resolved with definitive guidance and new hot topics will emerge. It is intended that all professionals with expertise in tissue banking will be involved in the challenge of evaluating these hot topics using the tools established in the Euro-GTPs website, and that the European Association of Tissue Banks (EATB) coordinates this process. In this chapter, some of the most significant hot topics are identified but hot topics are not exclusive to this chapter. Guidance to select adequate air quality during processing is another example of a hot topic still under discussion for improvement. The hot topics addressed in this chapter are discussed below.

4.3.1. Malignancy

According to EU Directives for tissues and cells, a history of malignancy can be considered an absolute exclusion criterion except for

primary basal cell carcinoma, *in situ* carcinoma of the cervix and some appropriately evaluated grade-I and -II primary tumours of the central nervous system according to the World Health Organization (WHO) classification (see Table 4.1)[6]. It is important to properly evaluate malignancy gradation in the central nervous system (CNS), including a complete histological exam and not just a simple biopsy, taking into account possible heterogeneity of the mass.

Donors with malignant diseases can be evaluated and considered for cornea donation except for retinoblastoma, haematological neoplasm and malignant tumours of the anterior segment of the eye. Any cornea for human application that is not limited to the non-vascularised part of the cornea, such as the limbus or limbal cells, cannot be considered to be covered by this exclusion.

In the European Directorate for the Quality of Medicines & HealthCare (EDQM) *Organ Guide*, a detailed risk analysis was undertaken regarding 'quantification of risk' according to the type of malignancy and time without symptoms to allow the possibility of not rejecting organs suitable to be transplanted with a 'limited' risk of transmission or to be transplanted in recipients with a high risk of death without having an organ transplant.

The *rationale* for this approach is the life-saving function of the organs in comparison with the generally non-life-saving nature of tissue transplants as well as the severe shortage of potential organ donors compared with the significantly larger pool of potential tissue donors.

This hot topic merits consideration of malignancies also for tissue donation by taking into consideration:

a. type of malignancy and time free of disease (as in the *Organ Guide*);

b. type of processing, manipulation and validation of the number of living cells in the final product and consequent risk of transmission (e.g. very low/negligible risk associated with decellularised heart-valves, decellularised skin, freeze-dried bone, and irradiated bone);

c. tissues that can be considered equivalent to organs in terms of life-saving capacity and with a very limited pool of suitable donors (e.g. paediatric heart valves).

This evaluation should be completed with an accurate risk assessment by a qualified and well-trained medical director focusing on the type of malignancy, course/recurrence, treatment, effect on the type of tissue to be recovered, and the processing that will be done. Health Authorities should review validations and risk assessments to authorise these exceptions to the generic rule. Notwithstanding this guidance, EU member states, in accordance with the Directives on tissues and cells, must consider malignancies to be an absolute exclusion criterion except for corneas (as described above).

It is important to evaluate conditions such as cirrhosis or serious gastrointestinal disorders that could result in an increased risk of malignancies, as well as to acknowledge the malignancies that are not detected when obtaining tissues for older donors without a thorough autopsy inspection, particularly, for example, prostate malignancy in donors aged >70 years.

Table 4.1. WHO grading of tumours of the central nervous system

From Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. World Health Organization, *Classification of Tumours of the Central Nervous System*. IARC, Lyon, 2007 [6]

	I	II	III	IV
Astrocytic tumours				
Subependymal giant cell astrocytoma	•			
Pilocytic astrocytoma	•			
Pilomyxoid astrocytoma		•		
Diffuse astrocytoma		•		
Pleomorphic xanthoastrocytoma		•		
Anaplastic astrocytoma			•	
Glioblastoma				•
Giant cell glioblastoma				•
Gliosarcoma				•
Oligodendroglial tumours				
Oligodendroglioma		•		
Anaplastic oligodendroglioma			•	
Oligoastrocytic tumours				
Oligoastrocytoma		•		
Anaplastic oligoastrocytoma			•	
Ependymal tumours				
Subependymoma	•			
Myxopapillary ependymoma	•			
Ependymoma		•		
Anaplastic ependymoma			•	
Choroid plexus tumours				
Choroid plexus papilloma	•			
Atypical choroid plexus papilloma		•		
Choroid plexus carcinoma			•	
Other neuroepithelial tumours				
Angiocentric glioma	•			
Chordoid glioma of the third ventricle		•		
Neuronal and mixed neuronal-glial tumours				
Gangliocytoma	•			
Ganglioglioma	•			
Anaplastic ganglioglioma			•	
Desmoplastic infantile astrocytoma and ganglioglioma	•			
Dysembryoplastic neuroepithelial tumour	•			
Central neurocytoma		•		
Extraventricular neurocytoma		•		
Cerebellar liponeurocytoma		•		
Paraganglioma of the spinal cord	•			
Papillary glioneuronal tumour	•			
Rosette-forming glioneuronal tumour of the fourth ventricle	•			
Pineal tumours				
Pineocytoma	•			
Pineal parenchymal tumour of intermediate differentiation		•	•	
Pineoblastoma				•
Papillary tumour of the pineal region		•	•	
Embryonal tumours				
Medulloblastoma				•
CNS primitive neuroectodermal tumour (PNET)				•
Atypical teratoid / rhabdoid tumour				•
Tumours of the cranial and paraspinal nerves				
Schwannoma	•			
Neurofibroma	•			
Perineurioma	•	•	•	
Malignant peripheral nerve sheath tumour (MPNST)		•	•	•
Meningeal tumours				
Meningioma	•			
Atypical meningioma		•		
Anaplastic / malignant meningioma			•	
Haemangiopericytoma		•		
Anaplastic haemangiopericytoma			•	
Haemangioblastoma	•			
Tumours of the sellar region				
Craniopharyngioma	•			
Granular cell tumour of the neurohypophysis	•			
Pituicytoma	•			
Spindle cell oncocytoma of the adenohypophysis	•			

4.3.2. Epidemiological data used to assess sexual-risk behaviours, tattoos, and incarceration

Questionnaires for risk assessment of behavioural risks are based on Centers for Disease Control and Prevention (CDC) recommendations. Some of the answers should be evaluated on the basis of a risk assessment to accept or reject, and some questions can be kept on the basis of epidemiology data.

4.3.3. Transplantation with xenografts

The current situation is based on conformity to Annex I of Directive 2006/17/EC [1], where it lists '1.1.13. Transplantation with xenografts' as a general exclusion criterion for donors. The EU Directive does not provide a definition for xenografts, and there is no *rationale* provided to support transplantation of a xenograft as a risk to the recipient. Dictionaries agree that 'xenograft' refers to 'a graft of tissue taken from a donor of one species and grafted into a recipient of another species' [2]. Xenografts are used every day in surgical procedures and are medical products derived from non-human animals. The directive opens this annex with the following statement preceding the list of exclusion criteria, 'Unless justified on the basis of a documented risk assessment approved by the RP as defined in Article 17 of Directive 2004/23/EC, donors must be excluded from donation if any of the following criteria applies', which allows for written justification to override this criterion.

Considerations for assessment of risk and its mitigation can include whether the transplant of a medical product derived from a non-human animal contains 'live' cells, tissues or organs, or uses these live cells during manufacturing. If manufacture of the product utilises only non-viable materials from a non-human animal species, risk is mitigated if the materials are subjected to pathogen inactivation procedures and the product can be considered safe [1].

Confusion remains regarding the universal definition for a xenograft *versus* the meaning of a xenotransplantation product (also known as a 'xenogeneic cell-based medicinal product') [3]. The definition of

xenotransplantation is any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues, or organs from a non-human animal source, or (b) human body fluids, cells, tissues or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs. Development of xenotransplantation is, in part, driven by the fact that the demand for human organs for clinical transplantation far exceeds the supply [1, 9].

Medicinal products such as bovine bones for orthopaedic reconstruction and porcine heart valves for cardiac replacement meet the definition of a xenograft comprising non-viable materials from a non-human animal and, therefore, should not qualify as general exclusion criteria for donation [1, 3]. These animal materials are subject to several inactivation procedures that minimise infection risk, and they do not contain viable cells.

A documented risk assessment is expected to be made if a donor has a history of having been transplanted with a xenograft.

4.4. Physical evaluation of donors

Physical evaluation is required for each donor, and should be designed to detect any physical signs implying a risk for transmissible disease. It should be carried out before procurement and must be documented. The physical examination results in rejection of 5% of deceased donors, thereby demonstrating its importance [4].

Information related to disease, lesions not known previously (tumours, skin lesions), diseases or treatments not mentioned by the donor or the family (e.g. scars) permit the professionals carrying out donor selection to return to the donor historian for clarification. Signs that imply risk related to donor social behaviour and lesions or fractures related to the cause of death that can totally or partially contraindicate the donation (e.g. open fractures) should be detected and recorded.

A sample tissue donor physical assessment form provided by the American Association of Tissue Banks can be found in Appendix 6.

Risks to look for should include signs of:

a. systemic disease:
   i. active malignancy (suspicious skin lesions);
   ii. malnutrition, multiple deformities.

b. bacterial or viral infection:
   i. recent receipt of a live vaccination (vaccination site infection, scabs, lesions);
   ii. recent receipt of a tattoo, body piercing or acupuncture where non-sterile instruments were used (shaved area, redness, swelling, scabbing may require further investigation to assess risk);
   iii. skin lesions such as a rash, petechiae, skin ulcers, blue/purple or grey/black lesions, shingles;
   iv. oral lesions such as ulcers or thrush;
   v. enlarged lymph node(s);
   vi. icterus, hepatomegaly.

c. high-risk behaviour (related to HIV infection or viral hepatitis):
   i. injectable drug use (non-medical injection sites);
   ii. inspection of tattoos (for hidden injection sites, also to assess extent and location of the tattoo);
   iii. genital or skin lesions or trauma indicative of a sexually-transmitted disease (e.g. evidence of anal intercourse, insertion trauma, peri-anal or herpetic lesions, syphilitic chancres or other lesions).

d. trauma:
   i. fractures, avulsions, lacerations or abrasions that may affect (contaminate, compromise integrity) the tissue to be recovered;
   ii. internal trauma that can cause cross-contamination between cavities (e.g. injury to the bowel, penetrating or crushing injuries).

e. cleanliness of the body, the condition in which the body was found (this can also relate to increased risk for contamination/cross-contamination).

f. scars (surgical, scarification); if findings do not match the donor's history, further investigation may be required.

Each donor (adult or child) must be thoroughly examined following established protocols, 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. No finding suggestive of possible risk should be left unresolved.

For living donors, a physical examination should be undertaken to ensure 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 physical examination.

In all such cases, visual examination of the body by procurement staff may be necessary during early, initial screening if adequate information regarding the condition of the body cannot reliably be obtained verbally.

4.5. Additional information

All relevant additional information should be collected and assessed for risks to the safety and quality of tissues and cells before the donation is released for human application. Additional information might be obtained from:

a. an autopsy report;

b. the general practitioner (if not yet consulted);

c. results of laboratory tests at the donor hospital (microbiology, chemistry, haematology, virology, urinalysis, toxicology and/or pathology);

d. radiography/magnetic resonance imaging/computed tomography;

e. surgical records;

f. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology).

4.6. Considerations for evaluation of paediatric donors

Special screening considerations are required for paediatric donors. If the child is 18 months old or younger, or has been breastfed in the 12 months before death, the birth mother should be evaluated for risks associated with HIV, HBV, HCV and HTLV. Other diseases that can be transmitted vertically from mother to foetus may also be relevant, such as malaria or Chagas disease. Special testing may be needed if a risk is deemed relevant. A child's immune system is only in development, so protective antibodies may not yet have been produced against an infection, thereby increasing the risk of hidden infections in child donors.

In the EU, Directive 2006/17/EC stipulates that children aged under 18 months born to mothers 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.

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

4.7. References

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Related documents:
Appendix 5 – Sample donor assessment form;
Appendix 6 – Sample tissue donor physical assessment form

Chapter 5

Donor testing

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 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. Controls include:

- selecting a qualified laboratory that will undertake testing following good laboratory practice;
- ensuring the validity of any donor blood specimen collected for testing of infectious diseases;
- use of appropriately validated tests for infectious diseases;
- providing well-written standard operating procedures (SOP) 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 a Responsible Person (RP) must keep informed of advances in testing technology for infectious disease (e.g. third- and fourth-generation antigen and antibody tests, nucleic acid amplification technique [NAT], etc.).

5.2. General concepts

Tissue establishments must ensure that all donations of human tissues and cells are subjected to those biological tests mandated by national legislation.[1]

SOP should be in place that define the criteria for acceptance or rejection of tissues and cells based on those test results.

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 used for testing of donor samples has been authorised by the appropriate authority to carry out such testing.

5.3. Quality of donor samples

Manufacturers of testing kits for infectious diseases typically publish specific sample requirements for each sample. Personnel of procurement organisations and tissue establishments involved in collecting, storing or testing donor samples must be aware of these requirements to ensure optimised test performance. If inadequate or adulterated samples are provided and tested, test results may not be valid which increases the risk of donor-related transmission of infectious diseases.

Donor-related conditions that could affect the quality of a test specimen must also be taken into account. Infectious disease test results may be invalidated by haemodilution or plasma-dilution if a qual-

1 In EU member states, Annex II of Directive 2006/17/EC, amended by Directive 2012/39/EU, stipulates mandatory laboratory tests and general testing requirements for living and deceased donors of tissues and cells.

ified, pre-transfusion or pre-infusion sample is not available (see section 5.3.2). Haemolysis can also affect test results.

The ability to detect antibodies against viral agents can be impaired if the donor has received immunosuppressive treatment. In this case, NAT assays can prove valuable because detection of nucleic acids is not affected by immunosuppressive therapy. The underlying condition requiring immunosuppression will demand further assessment because it in itself may constitute an independent reason for deferral. If any of these donor-related conditions exist, they must be documented in the donor record and evaluated by a RP before release of tissues or cells for clinical application.

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

All personnel involved in any stage of the testing process must be fully trained. Testing must be carried out on the serum or plasma of the donor according to the specification laid out by the manufacturer of the test kit. Testing must not be undertaken on other fluids or secretions, such as aqueous or vitreous humour, unless validation has been done to ensure test validity on that sample. In the case of a neonatal donor (i.e. age ≤1 month), the required tests should be carried out using a blood sample from the donor's birth mother.

Donor sample collection and manufacturer's test instructions must be followed with regard to:

a. the type of collection tube (no anti-coagulant or a specific anti-coagulant) required for the test being carried out;

b. sample storage and transport conditions post-collection, which can include centrifugation and/or separation within time-limits or specimen refrigeration/freezing;

c. testing required to be carried out within a specified timeframe post-collection.

To ensure traceability at each stage of the testing process, all donor samples must be identified with a permanently affixed label that

contains information or references that link the sample and the laboratory test results to the donor. 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, etc.). It is good practice for the identity of all donor samples to be confirmed by a second person from the procurement team, and this confirmation process should be documented [1]. If any donor blood samples were drawn before death, they can be qualified for use, but there must be assurance that the patient identifier used for any such specimen is confirmed as coming from the donor by appropriate labelling so mix-ups do not occur (i.e. carrying out testing of critical communicable diseases on the wrong patient) [1]. Other donor identification methods can be used, if validated, to ensure traceability [2]. Specimens of blood, serum or plasma collected at different times must not be mixed together for testing (e.g. a serum or plasma sample must not be physically combined with another serum or plasma sample collected at the same or 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 collecting, or otherwise obtaining, 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, otherwise the quantity of serum or plasma after centrifugation may not be sufficient to undertake all required tests for infectious diseases, or for repeat testing (if necessary). 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 evaluated before blood collection. Other considerations could include a donor with a high haematocrit (which could necessitate collection of extra tubes) and if a donor took (or was given) anticoagulant 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 3–4 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. Under normal circumstances, 2–3 full blood tubes should be sufficient for all types of testing.

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 anticoagulant, this liquid or powder requires that a completely filled tube be gently mixed by slowly inverting the tube 5–10 times immediately after collection [3].

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 among tests [1]. In all cases, qualified transport containers and validated shipping procedures must be used when sending donor samples to a testing laboratory.

The facility receiving any donor sample for testing should have 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 a 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.

It is important to collect blood samples without untoward delay after death to avoid sample characteristics that could cause false-positive test results (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) [1].

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, <7 days after donation. For practical reasons, collection of a sample from an allogeneic bone marrow stem cell or peripheral blood stem cell donor can occur within 30 days before donation, taking into account that re-testing at the time of donation will be informative, but without there being a point-of-return when irreversible measures for pre-conditioning of the recipient had been initiated.

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, 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 the donor's dilution that might render any test result invalid 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). 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 7 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 has lost blood. 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 donors, 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 [1]:

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);
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, carrying NAT tests for the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and/or hepatitis C virus (HCV) may be more appropriate.

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

5.4. Testing laboratories

To meet the high expectations required for quality and safety, 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. Ideally, such laboratories should participate in external quality assessments, such as proficiency testing. It can be challenging for laboratory personnel in hospitals to maintain adequate proficiency if they are infrequently running assays to test for infectious diseases. Hospital-based laboratories may employ diagnostic test kits intended for use only in symptomatic patients because such tests are not considered difficult to carry out and do not require expensive equipment. Reference laboratories carry out infectious-disease testing routinely using high-throughput, automated equipment. Also, they employ technicians with specific skills for undertaking highly complex testing. In addition, they use test kits designed for screening donors.

If additional biological assays are carried out for a donor of haematopoietic stem cells, the laboratory used should be accredited and participate in an appropriate external quality assessment programme [3].

Tissue establishments can undertake these testing protocols themselves or have a written agreement with any laboratory that carries out donor infectious-disease testing on their behalf. Tissue establishments should evaluate and select a testing laboratory on the basis of their ability to comply with regulatory requirements and any other specific expectations of the tissue establishment (e.g. time-sensitive availability of test results). The tissue establishment should assess the laboratory's qualifications, and also the test kits and procedures the laboratory intends to use. There must be evidence that good laboratory practice is being followed and that personnel are appropriately trained and experienced in the relevant testing procedures. 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 quality system.

5.5. Tests to be carried out

The donor screening assays selected must be validated and used in accordance with current scientific knowledge. If Conformité Européenne (CE)-marked test kits are available, they must be used (see Appendix 8: Validation of infectious disease screening assays for use with blood from deceased donors). As a minimum requirement, the biological tests listed below must be carried out on the serum or plasma of the donor according to the manufacturer's instructions for each test kit [1]:

a. HIV 1 and 2 – anti-HIV-1 and anti-HIV-2 (antibodies to HIV-1 and HIV-2);

b. HBV[2] – HBsAg (surface antigen) and anti-HBc (antibodies to core antigen) (total, i.e. IgG and IgM);

2 If anti-HBc (total, i.e. IgG and IgM) is positive (repeat reactive) and HBsAg is negative, further testing is necessary to determine hepatitis status in the

c. HCV – anti-HCV (antibody to HCV);

d. syphilis[3] – a treponemal-specific test or a non-specific treponemal test can be used.

Testing for HTLV-I antibodies must be undertaken for donors living in or originating from high-prevalence areas, or with sexual partners originating from those areas, or if the donor's parents originate from those areas [4, 6].

The minimum requirement for donor testing for viral infectious agents is antibody detection for HIV 1/2, HBV, HCV, and human T-cell lymphotropic virus (HTLV-1; when indicated), plus detection of antigen for HBV. Testing the birth mother is necessary if the donor is aged ≤18 months, or if the donor has been breastfed <12 months before donation.

5.5.1. Additional tests

NAT assays are more sensitive and deceased donors cannot be retested after 6 months. Hence, serious consideration should be given to also carrying out NAT testing for HIV, HBV and HCV. Considerations that support the use of NAT assays for each deceased donor include the following:

donor. Such determination usually involves anti-HBs, anti-HBc IgM (only), and/or NAT for HBV (see section 5.5.1). In these circumstances, risk assessment must be carried out and documented by the person responsible in the tissue establishment, who will determine donor eligibility and tissue release for clinical use. This strategy also applies when tissue or cell distribution occurs from procurement directly to the clinical team responsible for human application or for direct use.

3 A validated testing algorithm must be applied to exclude active infection with Treponema pallidum. A non-reactive test for syphilis (specific or non-specific) can allow tissues and cells to be released. When a non-specific test is undertaken, a reactive result will not prevent procurement or release if a treponemal-specific (confirmatory) test is non-reactive. A donor whose specimen is reactive for a treponemal-specific test requires thorough risk assessment by a Responsible Person to determine donor eligibility for their donation to be released for clinical use.

- the medical and behavioural history obtained from a proxy for a deceased donor can be less reliable than collecting this information from a living donor;
- if the donation includes multiple tissue types that results in a large number of tissue grafts to be made available for many recipients, the potential risk is increased if the viral tests selected cannot detect early infection in a donor.

To optimise detection when using a NAT assay, the donor blood sample should be tested individually.

With regard to additional testing for donors originating from high-prevalence areas for specific diseases or whose sexual partners or parents originate from such areas, one should refer to international scientific evidence, such as that provided by the European Centre for Disease Prevention and Control [4, 6]. Additional testing that may be considered (depending on the donor's history and the characteristics of the tissues or cells donated) includes:

a. ABO group;

b. RhD (D antigen);

c. human leucocyte antigen (HLA);

d. diagnostic tests for malaria;

e. West Nile virus NAT;

f. antibodies to cytomegalovirus;

g. toxoplasma antibodies;

h. antibodies to Epstein–Barr virus (EBV);

i. antibody to *Trypanosoma cruzi* (the infectious agent for Chagas disease).

Results of blood cultures can be very useful tools to aid determination of bacteraemia in donors of tissues and cells. However, protocols for collection of blood cultures must be controlled appropriately, and such protocols may be applicable only if collected before death. Care should be taken regarding interpretation of results (see Chapter 8).

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

For living donors, initial infectious-disease testing is carried out at the time of donation or ≤7 days after donation when this is not possible. However, 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. However, there are additional considerations because the donor is available for more testing.

Test kits for infectious diseases are typically optimised for testing a sample from a living donor. In addition, more tests could be indicated because there may be unique risks of infectious disease pertinent to a profoundly immunosuppressed recipient of bone marrow or of similar types of haematopoietic allograft.

If tissues and cells of allogeneic living donors can be stored for long periods, repeat sampling and testing is required after 180 days. Under such circumstances, donor sampling for initial testing can occur ≤30 days before and 7 days after donation. If samples from a living donor undergo serology testing and are also tested by NAT for HIV, HBV and HCV, re-testing after a time interval is not required. Due to the increased sensitivity of NAT assays for the detection of recently acquired infections, testing all donors using this technology is highly recommended as standard practice. Re-testing is also not required if processing includes an inactivation step that has been validated for the virus(es) concerned.

For testing individuals involved in assisted reproduction technology (ART), see Chapter 25 at 25.4.

5.5.3. Testing of autologous samples

For autologous donors (whereby the tissues or cells removed are stored or cultured and transplanted back into the donor), carrying out the same serological tests as for allogeneic donors may be desirable. If an autologous donor's blood sample has not been appropriately tested or if a test is positive 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. However, this is only the case if appropriate storage can provide isolation/segregation to ensure there is:

a. no risk of cross-contamination with stored allografts;

b. no risk of contamination with adventitious agents;

c. avoidance of mix-ups due to misidentification (see Chapter 12).

SOP 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 unauthorised 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 if they 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

a positive infectious disease test result occurs. Other precautionary measures regarding reporting may include [5]:

- where manual systems are still used (although they are not recommended), virology analysis reports should be cross-checked to ensure that the transcription of test results has been confirmed by two independent assessors;
- 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;
- using clearly interpretable, computerised graphic symbols to highlight positive results;
- including the titre of antibodies and/or the related positivity threshold next to the viral negative/positive result;
- use of formal laboratory reporting structures and accreditation or certification pathways to improve quality standards;
- use of widely recognised international units of measurement.

5.7. Archived samples

If any donor sample remains after all required testing has been completed, it is desirable to freeze and store aliquots of serum and/or plasma. Therefore, archive samples may be used for several purposes: look-back involving a new infectious agent, development of more accurate or new tests, or if investigating a report of a serious adverse reaction in a recipient of tissues or cells. A documented risk assessment, approved by the tissue establishment's RP, should be carried out to determine the fate of all stored tissues and cells following the introduction of any new donor test that could reasonably be considered to affect safety or quality (see Chapter 2).

5.8. References

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3. Clinical and Laboratory Standards Institute (CLSI). Procedures for the handling and processing of blood specimens; approved guideline. Third edition, volume 24, number 38.

4. European Centre for Disease Prevention and Control, available at www.ecdc.europa.eu/en/Pages/home.aspx, accessed 27 May 2015.

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Related documents:
Appendix 7 – Sample haemodilution algorithm;
Appendix 8 – Validation of infectious disease screening assays for use with blood from deceased donors

Chapter 6

Procurement

6.1. Introduction

To ensure high standards of quality and safety during the procurement process for tissues and cells, it is recommended that a quality system be in place in the procurement organisation or the tissue establishment undertaking the process. This quality system must guarantee adequate training of all personnel involved, as well as written standard operating procedures (SOP) that require documentation for all stages of the process. Procurement professionals should take measures to ensure appropriate safety and quality parameters are in place.

Procurement of human tissues or cells can take place only after mandatory donor consent or authorisation requirements have been satisfied, as described in Chapter 3. Tissues and cells must also be packaged and labelled correctly (see Chapter 12), and then transported to the tissue establishment or clinical team for direct use, in accordance with established requirements.

Chapter 2 details quality-management expectations regarding the personnel, facilities, equipment, materials, procedures, and documentation that should be considered in regard to applicability for procurement activities. However, additional considerations with a focus on procurement activities are detailed in this chapter. Chapters

in Part B of this Guide describe requirements for the procurement of specific tissues, such as ocular (see Chapter 15), amniotic membrane (see Chapter 16), skin (see Chapter 17), cardiovascular (see Chapter 18) or musculoskeletal tissues (see Chapter 19), or when obtaining haematopoietic stem cells (see Chapter 21), or other cells (see Chapter 22) or assisted reproductive technology (see Chapter 25).

6.2. Personnel

Procurement activities must be undertaken by personnel with appropriate qualifications, training and experience. This includes successful completion of a comprehensive technical and/or clinical training programme involving the specific tissue or cell types to be procured.

Persons undertaking procurement must be made aware of the risks (as well as the potential consequences) if policies and procedures regarding procurement are not followed as directed in SOP and according to relevant legislation.

To promote compliance with donor selection criteria and procurement procedures, the tissue establishment (or the procurement organisation) must have a written agreement with each person, clinical team or organisation involved in carrying out procurements, as well as those collecting critical information used in donor selection. The written agreement must include 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 personnel employed by the tissue establishment (or procurement organisation) responsible for these steps (though expectations and responsibilities pertaining to procurement must appear in their job description).

Staff must also undergo a comprehensive training programme, including the broader ethical, legal and regulatory context of their work.

6.3. Facilities, equipment and materials

6.3.1. Facilities

Procurement activities must be authorised by the appropriate and competent Health Authority(ies). Each procurement event must take place in an appropriate facility and follow the technical procedures (see section 6.4. Procedures) that minimise bacterial or other contamination of procured tissues and cells. For reasons of privacy and control of contamination, access to the area where procurement takes place must be restricted, especially during the carrying out of procurement activities.

Donation of tissues and cells by living donors must take place in an environment that ensures their health, safety and privacy.

It is highly recommended that the facility where procurement takes place:

a. is of adequate size regarding floor space and work-tops that will be used;

b. is appropriately located to ensure cleanliness and privacy;

c. is furnished with sufficient and suitable lighting;

d. is in a good state of repair;

e. is free of pests;

f. provides a sufficiently clean or cleanable environment that will not contribute to contamination of the cells or tissues during procurement activities.

Before procurement, steps to minimise the potential for contamination must include cleaning all working surfaces with an approved disinfectant. The procurement area must also be cleaned appropriately post-procurement and this involves proper disposal of all biohazardous waste and sterilisation of all re-usable instruments. If a tissue establishment (or procurement organisation) uses the general cleaning services of the host facility to clean and/or sterilise the op-

erating room as well as surgical instruments, the procedures used by them must be inspected/validated.

It is recommended that the procurement area meet the criteria of a standard operating room:

a. ancillary areas used for preparation or entry to the procurement area should be reserved for this use and be cleaned according to a procedure that minimises the risk of introducing contamination;

b. rooms used for changing clothes, for preparatory washing (forearm and hand scrubbing) and personal hygiene should be easily accessible and appropriate for the number of procurement personnel;

c. rooms provided with plumbing and drainage systems should not directly communicate with procurement or equipment storage areas.

If this is not possible, risk assessment must be carried out and documented.

If the tissue establishment has its own procurement area, whenever possible, a controlled environment (such as exist in hospital operating rooms) should be used. Environmental control systems are necessary when procedures are undertaken in an open environment and should ensure at least a Grade D classification of the procurement area 'at rest'. Alternatively, if the area is not classified, a validated cleaning methodology must be applied. It should provide comfortable and safe working conditions (in terms of temperature and humidity), and have filtered ventilation with pressure gradients that direct air to circulate from the cleanest area in the room to areas of the lowest level of cleanliness. To qualify these controls, annual environmental and operational qualification procedures can be undertaken (e.g. filter integrity testing, air exchange and pressure measurements, airborne particle counts, temperature and humidity measurements). Instrumentation used for such measurements must be properly maintained and calibrated, and used according to the manufacturer's instructions.

If procurement of tissues (e.g. cornea) or cells must occur outside of a designated procurement area (e.g. mortuary, funeral home or, for a living donor, in their residence or hospital room), a secure, enclosed space must be prepared to limit (as far as possible) potential environmental contamination during procurement.

6.3.1.1. *Processing at the procurement stage*

Control of contamination and cross-contamination at the procurement site is typically less stringent than controls used in a processing suite. However, requirements cannot be bypassed if processing is carried out at procurement. In all cases, processing must be done under strict processing requirements, even if it is undertaken simultaneously with procurement or in the same room after procurement.

Processing includes shaping, cleaning, sizing and packaging after the tissue has been procured.

In any setting, microbiological safety during the procurement of tissues or cells must be considered. Due to specific contamination control and other quality-assurance requirements (described in more detail below and in Chapters 2 and 15–21), simultaneous undertaking of 'processing' steps during the procurement phase, or in the procurement area, is not recommended. The strict environmental controls (i.e. air quality Grade A if tissue and cells are exposed to the environment during processing steps (see Chapter 7) and monitoring requirements expected for 'processing' cannot be met by the sites at which procurement usually takes place (i.e. an operating room or an area of less stringent control).

If processing at the procurement site is unavoidable, its duration and extent should be limited to the minimum necessary, and an air quality Grade A environment (surrounded by, at least, air quality Grade D) for the processing steps is desirable (such as a laminar flow cabinet located in the operating room). Records supporting the qualification of the processing site must be available for inspection. If this level of control is not possible, an in-process (active) environmental monitoring method should be used; preferably, active air monitoring using

a viable particle counter and culturing method or, as a minimum control, microbiological settle plates can be used. Sample cultures of the tissues or cells procured should also be taken (see Chapter 8) 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 the:

a. type of tissues and cells procured;

b. various tissue and cell processing steps that will be used (e.g. none; exposure only to antibiotics; a validated inactivation method; or, a validated sterilisation method);

c. planned human application (as well as consideration of the immune status of the recipient, if applicable).

Selection of the use of suboptimal conditions must be supported by written justification and be authorised by Health Authorities.

6.3.1.2. *Risk assessment of the air quality of the procurement area*

The tissue establishment can apply a failure mode effects and analysis (FMEA) risk-assessment method to evaluate the risk of microbiological contamination of the tissue during processing (see Chapter 7, 15–19). Procurement will affect directly the risk assessment done at tissue processing. Hence, the air quality of the procurement area must be taken into account together with other processing factors to define the specifications of the processing of air quality.

Air quality of the procurement area will affect the microbiological safety of recovered tissues. The effect or influence of the air quality of the procurement area will be different depending of tissue to be retrieved:

a. Eye procurement: A classified area is not usually required because of the tissue type and recovery methodology (see Chapter 15);

b. Skin procurement: This is considered an aseptic procedure (see Chapter 17) in the same way as eye recovery. Despite this con-

sideration, any classified procurement area will improve tissue quality;

c. Cardiovascular and musculoskeletal procurement: Both tissues require sterile recovery methodology (see Chapters 18 and 19). The best recovery situations would be a class C or D procurement area. A non-classified recovery area will have the highest score;

d. Living donation (tissues that require sterile recovery): The same method as that for cardiovascular and musculoskeletal tissues is applied for these tissues (see Chapters 16 and 23) except for processes undertaken with a closed system.

Based on the risk tool for defining the air quality of the processing area (see Chapter 7), the updated risk tool shown in Table 6.1 below can be used to evaluate each particular tissue and process.

To specify an appropriate processing of air quality on the basis of the scores in the risk tool, the following scheme is recommended as guidance:

- <22: Class A with D background;
- 23–32: Class A with C background;
- >32: Class A with B background.

Table 6.1. Example of risk assessment for procurement of skin, eye, cardiovascular and musculoskeletal tissue (modified from EURO GTPs Hot Topics – www.eurogtps.com)

Factor		1	3	5	7	9	Score
Air quality of the procurement area (classified or non-procurement area)	Skin	Any classified procurement area	Non-classified procurement area				
	Eye	Classified or non-procurement area					
	Cardiovascular and musculoskeletal*	Class C procurement area	Class D procurement area			Non-classified procurement area	
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method (processing stage)		10% destructive testing in compliance with the *European Pharmacopeia*	Tissues preserved in culture medium (contamination is visible)	A biopsy of tissue tested from each individual graft processed	Pieces of tissue tested for each process run (<1% of the run)	Swabbing of final tissue	
Risk of contamination during processing		Tissue exposed to processing environment for <1 h	Tissue exposed to processing environment for 1–3 h	Tissue exposed to processing environment for 3–5 h	Tissue exposed to processing environment for 5–7 h	Tissue exposed to processing environment for >7 h	

* Also including living donation procurement under sterile conditions (e.g. amniotic membrane, heart valves).

Factor		1	3	5	7	9	Score
Use of antimicrobial agents		Agents with validated reduction of microbial contamination (by >3 logs)	Agents with published evidence of effective reduction of microbial contamination (85% glycerol for skin processing)	Validated treatment using an 'antibiotic cocktail'	Fluid storage and conditions in final container do not promote microbial growth	Fluid storage and conditions promote microbial growth (temperature >10°C)	
Risk of transfer of contaminants at transplantation	Tissue type	Acellular	No vital cells	Vital cells but no vessels	Vital cells and minor vessels	Fully vascular with vital cells	
	Type of use	Temporary superficial coverage <3 weeks	Durable superficial coverage >3 weeks	Durable implant in a poorly vascularised site	Small durable implant in a well vascularised site	Large durable implant in a well vascularised site	

6.3.2. Equipment and materials

Materials (e.g. disposables and reagents) and equipment (e.g. instruments, devices, packaging, 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). Qualified sterile instruments, CE (Conformité Européenne)-marked devices (where available) and sterile single-use materials (e.g. drapes, gloves, fluids, etc.) 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 to ensure successful activities. This must include visual inspections and scheduled calibration of devices with a measurement function. Routine annual maintenance inspections (qualification procedures) of equipment used for procurements are encouraged and a re-qualification assessment is advised whenever repairs or modifications have occurred. Procurement personnel must receive appropriate training, supported by records, on the proper use of equipment.

Use of disposable instruments for procurement is recommended, whenever feasible. When re-useable instruments are utilised, compliance with a validated cleaning, disinfection and sterilisation process for removal of infectious agents must be routinely used and these steps should be supported by documentation for each event. A system must be in place that allows tracing of 'critical' equipment and materials to each tissue or cell procurement event, and to the donor.

In EU member states, critical reagents and materials must meet requirements and specifications and, if 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 personal protective equipment (clothing) appropriate for the type of procurement. Usually, this will extend to being scrubbed and involves wearing a sterile gown, sterile gloves, glasses, 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. *Packaging, containers and labelling*

Immediately after procurement, tissues and cells must be packaged so as to minimise the risk of environmental contamination. Labelling must be appropriate to ensure the traceability of tissues and cells. Labelling must be resistant to storage conditions to avoid the loss of identification of tissues and cells. Guidance for this critical step is provided in Chapter 12 for all stages of the donation process, from procurement to distribution, including management of packaging material.

6.4. Procedures

Technical SOP for procurement must be in place based on the requirements of Health Authorities, the recommendations laid out in this Guide, and the expectations of the tissue establishment or end-user needs. These SOP must outline the correct steps to be taken for each stage of procurement and, indeed, all stages of the donation and transplantation process. Procedures that ensure contamination control must be used, such as use of sterile techniques, material and equipment (like those used during surgical procedures on patients), and personnel conducting the procurement to be attired appropriately (see sections 6.2 and 6.3.2). Periodic review of technical SOP by a responsible person must be undertaken and updates may be necessary due to scientific and technical progress. Procedures must be authorised and appropriate for the type of donor and the type of tissue or cells procured and must be standardised.

SOP must be readily accessible so procurement personnel can follow required steps, including:

a. verification of the donor's identity and what constitutes evidence of donor (or the donor family's) consent or authorisation (see Chapter 3);

b. assignment and appropriate use of a unique identifier/code (see Chapter 13);

c. knowledge of selection (risk) criteria required for donor assessment, including physical examination of the donor (see Chapter 4);

d. knowledge and assessment of the laboratory tests required for donor acceptance (see Chapter 5);

e. steps that minimise the risk of microbiological contamination during procurement (see this chapter as well as Chapters 2 and 15–21);

f. procurement steps that protect the properties of the tissue and cells required for clinical use (see this chapter and Chapters 15–21);

g. for deceased donation, how to reconstruct the donor's body so it is as similar as possible to its original anatomical appearance;

h. considerations for packaging, labelling and transportation of procured tissues or cells to the tissue establishment or, in the case of direct distribution, to the clinical team responsible for their human application or direct use (see this chapter and Chapter 12);

i. considerations for collecting, packaging, labelling and transporting samples of donor blood or donor fluid to the laboratory for testing (see this chapter, and Chapters 5 and 13);

j. procedures that protect the safety of the living donor (see Chapters 16 and 20–25).

In addition, the tissue establishment (or procurement organisation) is expected to have procedures in place to notify, without delay, other tissue establishments or the appropriate and competent Health Authority(ies) of all relevant available information regarding (see Chapter 14):

a. knowledge of deviations from approved procedures that occurred or that are suspected to have occurred; and/or

b. any serious adverse reaction in a living donor that may influence the quality and safety of the tissues and cells procured.

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

Additional procedures and policies that minimise the risk of microbiological contamination during procurement must be considered, including those listed below (see also Chapters 15–22):

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

b. preparation of the donor's skin must follow the recommended standards of practice used for surgical patients and must occur at the beginning of procurement using an appropriate anti-microbial agent designed for this purpose;

c. the procedure for skin disinfection should account for the elimination of bacterial spores as well as vegetative micro-organisms and therefore include suitable disinfectants, their concentrations and durations of exposure;

d. before use, materials and equipment to be used must be visually inspected by procurement personnel to ensure they meet specifications (e.g. sterile, seals not broken, equipment functioning as expected, etc.);

e. for deceased donation, it is advisable to procure tissue before the autopsy takes place but, if this is not possible, detailed procedures must be written to address the increased potential for contamination when procurement takes place after autopsy.

Procedures must be written to accommodate procurement steps that protect certain properties of the tissue and cells required for their ultimate clinical use. These are described more fully in Part B of this Guide (tissue-specific chapters), but generally include:

a. procurement time-limits (after death): the general rule is 24 h if the body has been refrigerated in the first 6 h after death or 15 h if the body has not been refrigerated;

b. preservation of important anatomical structures and other tissue or cell characteristics;

c. temperature requirements during storage and transport to the next destination;

d. avoidance of delays in transport due to time-limits in place for processing after procurement.

Efforts should be made to ensure that procurement procedures do not substantially affect 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 if funeral proceedings are planned (e.g. face, neck, arms, etc.).

Tissue establishments (or procurement organisations) must have procedures that address retention of records (i.e. archived procurement records, see Chapters 2 and 11).

6.4.1. Temporary storage and transport

Critical parameters related to maintaining environmental conditions (e.g. temperature, sterile packaging) must be controlled during temporary storage and transport of recently procured tissues and cells. Records to demonstrate compliance with specified storage conditions must be created and maintained unless temporary methods of storage and transport have been validated (periodic revalidation procedures are recommended).

Temporary storage must provide clearly separate and distinguishable areas for tissues and cells that remain in quarantine. To prevent mix-ups or cross-contamination, physically separate areas, storage devices or secured segregation within a storage device/unit (i.e. refrig-

erator, freezer) must be allocated and labelled prominently (including at least the minimum required information – see Chapter 12). Temporary storage areas or units for tissues and cells must be monitored (and alarmed, if necessary) and checked to ensure expected environmental requirements are being met.

Transport of recently procured tissues and cells should occur without delay using an approved courier. The courier or transportation service must maintain records of pick-up and delivery, as well as the container's contents, so that the package is fully traceable. The procedures, materials and equipment used by procurement personnel to package and transport recently procured tissues and cells must be approved or provided by the tissue establishment or the clinical team who will receive them (see Chapter 12).

Evidence of the integrity of packages and their contents upon arrival at their destination must be documented and reported to the procurement organisation.

6.5. Documentation

Procurement is a critical activity, so descriptive documentation of the steps taken, the materials and equipment used, and identification of the personnel involved must be recorded and made available. Such records must be clear and legible, protected from unauthorised amendments, retained and readily retrievable throughout a specified retention period, and in compliance with data protection legislation. Procurement records must be as detailed as necessary to facilitate traceability, providing a complete history of the work undertaken and be capable of linking the records to the particular donor and tissue and cells procured. When tissues and cells are to be sent across national borders, consideration of possible language barriers should be addressed and a common language agreed upon for all documentation 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 13). This coding

must be in place to ensure an effective and accurate system capable of tracking tissues throughout all handling stages, including an identifiable link to the procurement steps. For each donor, there must be a record containing the donor's identity (i.e. first 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 register maintained for this purpose.

Before the procurement of tissues and cells may proceed, an authorised person must confirm and 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 destination (including the clinical team responsible for human application or direct use in the case of living donors). This procurement report, at a minimum, must contain:

a. donor identification data (first name, family name, date of birth and sex, as well as how and by whom the donor was identified, or donor identification in case of an unrelated haematopoietic stem cell (HSC) donor);

b. the environmental conditions of the procurement facility (location or description of the physical area where procurement took place);

c. a list of observations during the physical examination of the donor's body (for a living donor, only when such an examination is justified);

d. a description and identification of procured tissues and cells, including samples for testing of infectious diseases;

e. the identification of the person who is responsible for the procurement session (including his/her signature);

f. date, time (where relevant, start and end times) and location of procurement;

g. type, volume, manufacturer and the lot/batch/serial number of reagents, additives and the tissue and cell transport solution(s) used;

h. the name and address of the tissue establishment or procurement organisation;

i. the 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;

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

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

Related documents:
Appendix 9 – Sample form to assess the suitability of the working environment;
Appendix 10 – Sample donor identification form

Chapter 7

Processing and storage

7.1. Introduction

'Processing' means all operations involved in the preparation, manipulation, preservation and packaging of tissues or cells intended for human application. Storage occurs 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 during storage and that cross-contamination or loss of traceability is avoided.

The opportunity to process tissues and cells brings great advantages. The aims of processing include:

a. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use doses 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 or adverse reactions by removing those elements that are not necessary for the success of the transplant and the inactivation of microbes or even sterilisation in circumstances where cell viability is not required.

Processing includes a range of activities such as (but not limited to) cutting, grinding, shaping, centrifugation, soaking in antibiotic or

antimicrobial solutions, sterilisation, irradiation, separation, concentration or purification of cells, filtering, lyophilisation, glycerol preservation, freezing, and cryopreservation.

Although it brings great benefits, processing can also introduce risks. The potential risks include microbial contamination from the environment or cross-contamination from other tissues or cells, mix-ups in identification or labelling errors or having a detrimental impact on those characteristics of the tissues or cells that render them clinically ineffective. For these reasons, all the necessary steps must be carried out within a comprehensive quality management system, must be documented in standard operating procedures (SOP) and must be thoroughly validated to demonstrate that the quality and efficacy of the final product has not been compromised and that contamination or cross-contamination have not been introduced during processing.

This chapter provides generic guidance on the processing and storage of tissues and cells carried out by tissue establishments or cell establishments (referred later as 'tissue establishment'). Further, more specific, guidance is provided in Part B of this Guide.

7.2. Acceptance criteria (receipt at the tissue establishment)

Each tissue establishment must have a documented policy and specifications against which each consignment of tissues and cells (including blood samples from donors) 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. When the procured tissues or cells arrive at the tissue establishment, there must be documented verification of the consignment. Documents must be completed and cover 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 European Union [EU] countries, the requirements of Annex IV of 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 as well as the tissues and cells received, together with any accompanying samples, should be examined to ensure that they have not been damaged in 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 or incidents relating to storage;

c. transport conditions (unless a validated transport method has been used) and storage temperature;

d. identification of the donor (donation number);

e. description of the tissues or cells;

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 tissue establishment must ensure that the tissues and cells received are quarantined and stored in a defined, separated and adequate location and 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 tissue establishment include:

a. consent/authorisation, including the purpose(s) for which the tissues and cells may be used (i.e. therapy/transplant, education or research, or both therapeutic use and research/education) 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 history (see section 6.5);

c. for allogeneic donors, a properly documented review of the complete donor evaluation against the appropriate selection criteria by an authorised and trained person;

d. in the case of cell cultures intended for autologous use, documentation of the possibility of medicinal allergies (such as to antibiotics) of the recipient.

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

The tissue establishment 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.

The tissue establishment should sign an agreement that defines the responsibilities of each party in the transport of the tissues and cells to the tissue establishment if the material is not being transported by their own personnel. Such transportation should be direct, without intermediate stops when possible, to ensure the safety and maintenance of the temperature conditions of the tissues and cells and prevent unauthorised access.

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

7.3. Coding

Tissue establishments must ensure that human tissues and cells are correctly identified at all times. Upon receipt of the tissues and cells, the tissue establishment should assign a unique identification code to the material if this has not already been done at procurement. This

code can then be extended to identify the different products and batches of tissues or cells obtained during processing and storage (for further information on traceability, see Chapter 13).

Tissues and cells should be labelled at all stages of processing and storage (see Chapter 12 for further guidance on labelling). The label must include at least the following information:

a. unique identification;

b. identification of the tissue establishment;

c. type and characteristic of the product;

d. batch number (if applicable);

e. recipient name (if applicable).

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

7.4. Processing

7.4.1. General

Tissues and cells should be appropriately processed and preserved for clinical use. Tissue establishments must include all processes that affect quality and safety in their SOP.

Tissue establishments must ensure that the equipment being used, the working environment, process design, and validation and control conditions are in compliance with established quality and safety requirements (see Chapter 2).[1] Each step of processing must be carried out under defined conditions to guarantee the quality of tissues and cells as well as the safety of personnel and recipients.

If a tissue establishment entrusts one of the stages of processing to a third party, a written agreement is needed between the tissue establishment and the subcontracted party. The tissue establishment shall

1 In the EU, these requirements are laid down in Directive 2006/17/EU and 2006/86/EC. Other useful standards may be found in the EQSTB guidance.

evaluate and select third parties on the basis of their ability to meet the established standards.

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

The reagents used in preservation and processing should be of an appropriate grade for their intended use, be sterile (if applicable) and comply with existing national regulations. Use of antibiotics during procurement, processing and preservation should be avoided if possible or at least be justified by the institution. Whenever possible, reagents used for procurement, processing and preservation should be approved for human use and should be CE (Conformité européenne)-marked. Reagents not approved for human use may be used if reagents or procedures that include the reagent have been approved by national authorities or have been established in medical literature to be acceptable for the purpose specified. The origin, characteristic conditions for storage (physical, chemical, microbiological) and expiry dates of reagents should be monitored and recorded. Reagents should be used in a manner consistent with the instructions provided by the manufacturer. Critical reagents and consumables should have written specifications describing, if applicable:

a. description of the materials, including:
   i. the designated name and the internal code reference;
   ii. the reference (if any) to a pharmacopoeia monograph;
   iii. the approved suppliers and, if possible, the original manufacturer of the products;
   iv. a specimen of printed materials.
b. directions for sampling and testing, or reference to procedures;
c. qualitative and quantitative requirements with acceptance limits;

d. storage conditions and precautions;

e. the maximum period of storage before re-examination.

7.4.2. Processing methods

Processing methods must not render the tissues or cells clinically ineffective or harmful to the recipient. They should be designed to ensure the safety and biological functionality of prepared tissues and cells. Processing methods should be validated (see the general text on validation in Chapter 2 and section 7.4.3. below).

The processing procedures must undergo regular critical evaluation to ensure that they continue to achieve the intended results.

A written change control procedure must be followed before any significant change is implemented in processing. A change control procedure ensures that the change is justified, is documented, and will not affect the quality of the product. All relevant persons should be involved in evaluation of the change.

As a minimum, the following aspects should be evaluated:

a. significance;

b. effect on quality;

c. need to update SOP;

d. need to re-validate the process;

e. effects on quality control (QC) analyses;

f. need to inform regulatory authorities;

g. need to train personnel;

h. effect on risk analyses.

Pooling of donors during processing must be avoided. The only exception is if it has been demonstrated to be the only way of providing clinically effective tissues or cells.

7.4.3. Processing validation

If processing is carried out according to good manufacturing practice (GMP), the processing validation must be done according to GMP guidelines. In the EU, Directive 2006/86/EC allows for validation studies to be based on any of the following:

a. studies undertaken by the establishment itself;

b. data from published studies;

c. for well-established processing procedures, retrospective evaluation of the clinical results for tissues and cells supplied by the establishment.

Where validation is based on studies carried out by the establishment itself, reports should include at least the following elements:

a. a validation plan that specifies the critical parameters to be assessed and the acceptable result thresholds for these parameters;

b. a documented methodology;

c. all results obtained, described clearly and with relevant interpretation;

d. a signed declaration of validation acceptance or rejection by the Quality Manager (QM) or the Responsible Person (RP).

Validation studies should be carried out by applying 'worst case' scenarios. The equipment used for validation studies should be fully qualified, and measuring devices should be calibrated to traceable standards. Validation experiments should be repeated at least thrice, though this will depend on the degree of variability in the data, to ensure reliably repeatable results. For an example of a validation study, see Appendix 7.

Where validation is based on data from published studies, the relevant publications should be filed as part of the validation record. In this case, the tissue establishment should demonstrate that they can effectively reproduce the published process with the same results in their facility (operational validation). Copies of the relevant SOP and the results of the operational validation should be provided to demon-

strate that the process is equivalent to that applied in the published study(ies).

Where specific steps have been modified or adapted, separate validation should confirm that these changes have not invalidated the method. There should be a signed declaration of validation acceptance or rejection by the QM or RP.

If validation is based on retrospective evaluation of the clinical results for tissues or cells supplied by the establishment (i.e. for well-established processing procedures), data should be collected and analysed that include the number of tissues or cells implanted following processing by the method under consideration, and time period during which these implantations occurred. It should be demonstrated that, where a vigilance system was already in place at the time, clinical users were informed of the procedure for reporting adverse reactions. There should be a signed declaration of validation acceptance or rejection by the QM or RP.

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, site-specific validation studies should be completed to demonstrate the log reduction achieved by the process.

Subsequent to process validation and during routine processing, tissue establishments should monitor tissue and cell quality to ensure a state of control is maintained throughout the processing part of the product lifecycle. This will provide assurances for the continued capability of the process and 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 constitutes a critical element of ongoing process verification. For new or significantly changed processes, a project of close clinical outcome monitoring should be agreed with clinical users.

7.4.4. Requirements of processing facilities

Facilities for aseptic and clean, non-sterile processing must be dedicated to this activity, and must be designed, qualified and monitored to ensure that air quality is appropriate for the process being carried out. An international standard, such as the GMP guidelines and/or ISO 14644-1 Cleanrooms and associated controlled environments, should be followed regularly to achieve the appropriate air quality.

Processing of tissues and cells should be carried out in clean areas. In these areas, the concentration of airborne particles (viable and non-viable) is controlled to specified levels. Each processing operation requires an appropriate level of environmental cleanliness in the operational state to minimise the risks of particulate or microbial contamination. According to GMP, four Grades can be distinguished:

- Grade A (normally provided by a laminar air flow hood). Laminar air flow systems should provide a homogeneous air speed in a range 0.36–0.54 m/s (guidance value) at the working position in open, clean-room applications. Maintenance of laminar flow should be demonstrated and validated. Unidirectional air flow and lower velocities may be used in closed isolators and glove boxes;
- Grade B (the background environment for the Grade A zone if tissues or cells are processed according to GMP). The risk-assessment tool for defining the air quality can be used to select the background (Table 7.2);

- Grade C and D (clean areas for carrying out less critical stages in accordance with a documented risk assessment of the processing activities).

Comparison of different clean-room standards is shown in Table 7.1.

Table 7.1. Comparison of different classification standards for clean rooms (references: ISO Standard 14644-1, GMP Annex 1, Federal Standard 209E, Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones)

Class	Maximum particles/m³						FED STD 209E equivalent	GMP equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm		
ISO 1	10	2.37	1.02	0.35	0.083	0.0029		
ISO 2	100	23.7	10.2	3.5	0.83	0.029		
ISO 3	1 000	237	102	35	8.3	0.29	Class 1	
ISO 4	10 000	2 370	1 020	352	83	2.9	Class 10	
ISO 5	100 000	23 700	10 200	3 520	832	29	Class 100	A
ISO 6	1.0×10^6	237 000	102 000	35 200	8 320	293	Class 1 000	
ISO 7	1.0×10^7	2.37×10^6	1 020 000	352 000	83 200	2 930	Class 10 000	B
ISO 8	1.0×10^8	2.37×10^7	1.02×10^7	3 520 000	832 000	29 300	Class 100 000	C
ISO 9	1.0×10^9	2.37×10^8	1.02×10^8	35 200 000	8 320 000	293 000	Room air	D

Whichever classification is applied, facilities should have:

a. floors, walls and ceilings of a non-porous material with smooth surfaces to minimise the shedding or accumulation of particles (viable and non-viable) that are easily disinfected;

b. temperature control;

c. for sterile processing, air passed through high-efficiency particulate air (HEPA) filters with an appropriate pressure differential between adjacent zones of different Grades (15±5 Pa) that should be documented;

d. a documented system for monitoring temperature, air supply conditions, particle numbers and bacterial colony forming units (environmental monitoring, see below);

e. a documented system for cleaning and disinfecting rooms and equipment;

f. a documented system for gowning and laundry;

g. adequate space for personnel to carry out their operations;

h. adequate space for storage of sterile garments;

i. access limited to authorised personnel.

Characteristics such as temperature and relative humidity are dependent upon the product and processing methods used. Parameter settings should not interfere with the defined cleanliness standard. For temperature and relative humidity, the generally accepted guidance values are 18±2 °C and 40–60%, respectively. Once the environmental temperature and relative humidity requirement have been set to guarantee the safety and quality of the product, staff comfort can also be taken into account. To minimise the risk of cross-contamination, a positive pressure in clean-room facilities should be created. The pressure differential between adjacent zones of different Grades should be 15±5 Pa with the maximal air pressure in the processing room. This forms a 'pressure cascade' which ensures that the air flows only outwards and limits the entry of contamination into the clean rooms. Specific pressure requirements for gene-therapy manufacturing are described in Chapter 24.

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], human immuno-

deficiency virus [HIV]). Required precautionary activities or need for a special containment facility (processing room having reduced air pressure relative to the adjacent rooms) should be determined by documented risk analyses. Risk analyses should consider risks for 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 (*Directive on the protection of workers from risks related to exposure to biological agents at work*).

7.4.5. Selecting the appropriate air quality for processing

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

a. a validated microbial inactivation or validated terminal sterilisation process is applied;

b. or, 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;

c. or, 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;

d. or, 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 (2006/86/EC).

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

The specification of the air quality of the processing facilities should be decided on the basis of the particular types of tissues or cells and the processing method that is being applied. Several criteria should

be taken into consideration, as shown in Table 7.2. Where the risk of tissue or cell contamination during processing is high, and the chances of any contaminants being transferred to the recipient are high, a more stringent air quality specification should be adopted. See also table 6.1 (Chapter 6) and the tissue-specific recommendations provided in Part B of this Guide.

Table 7.2. Criteria to be considered to determine the air-quality specifications of the processing facilities according to EuroGTP guidance

Criterion	Explanation
Risk of tissue or cell contamination during processing	Processes that are mostly 'closed' need a less stringent specification than those that involve hours of open processing.
Use of anti-microbials during processing	Some tissues, even though not terminally sterilised, can be treated with various anti-microbial agents. This diminishes the risks of transferring any environmental contaminants.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method.	If the only possibilities for final microbiological sampling are swabbing or testing of unrepresentative samples, the risk that environmental contaminants will be undetected is higher compared with processes in which 5–10% destructive testing of final products can be performed (see *European Pharmacopoeia*, Sterility, section 2.6.1).
Risk of transfer of contaminants at transplantation	Tissues that are minimally processed, cellularised, or containing 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 *vs* temporary) and site of transplantation affects the risk of transfer of contaminants.

7.4.6. Environmental airborne monitoring

Clean rooms and laminar flow hoods must be classified, requalified and monitored in accordance with EN ISO14644 and EU GMP Annex 1.

The maximum concentration of airborne particles for each Grade is given in Table 7.3.

Table 7.3. Maximum permitted concentration of airborne particles for each Grade by EU GMP Annex 1 Manufacturing of Sterile Medicinal Products

Maximum permitted number of particles per m^3 equal to or greater than the tabulated size				
	At rest [a]		In operation [b]	
Grade	0.5 µm	5 µm	0.5 µm	5 µm
A	3 520	20	3 520	20
B	3 520	29	352 000	2 900
C	352 000	2 900	352 0000	29 000
D	3 520 000	29 000	Not defined*	Not defined*

a The 'at rest' state is the condition in which the installation is installed and operating, complete with processing equipment but with no operating personnel present.
b The 'in operation' state is the condition in which the installation is functioning in the defined operating mode with the specified number of personnel working.
* If the limit of the number of particles is not defined, each tissue establishment should determine the limits according to a risk-assessment study.

7.4.6.1. *Classification of clean rooms and laminar flow hoods*

a. For classification of a clean area, required tests and acceptance criteria should be defined in the approved qualification protocol.

b. For particle count, the minimum number of measurement points (number of sampling locations = N_L) of the facility is calculated as the square root of its area in m^2 rounded to a whole number

up ($N_L = \sqrt{A}$). Measuring points should be distributed evenly and at the same height.

c. For classification purposes, portable particle counters with a short charge tube should be used to avoid loss of particles.

d. Qualification in operation may be carried out during routine or simulated operations.

e. For classification purposes in Grade A zones, a minimum sample volume of 1 m^3 should be obtained in each measurement site. In other cases, the minimum air sample volume per measurement point should be 2 L and the minimum sampling time should be 1 min for each point.

f. To reach the air Grades B, C and D, the number of air changes should be related to the size of the room as well as the equipment and personnel present in the room.

g. Samples with too-high particle counts should not be automatically rejected. A single measurement can be rejected due to equipment malfunction, failure of the procedure or because of exceptionally clean air, for the following reasons: the calculations will be repeated for the other points, at least three measurements will be taken into account in the calculations, no more than one measurement value will be omitted during calculations, and the cause of incorrect results will be documented and approved.

7.4.6.2. *Monitoring of clean rooms and laminar flow hoods*

a. For monitoring purposes, parameters for control of clean areas should be supported by data obtained during qualification studies.

b. Clean rooms and laminar flow hoods should be routinely monitored while in operation. Measuring points should be determined on the basis of a formal risk-analysis study and the results obtained during qualification of clean rooms.

c. Adequate warning and alarm limits should be set on the basis of the results of the monitoring of particles. If the limits are

exceeded, processing procedures should be modified and appropriate corrective actions taken.

d. Monitoring systems for airborne particles may include: independent particle counters, a sequential sampling system, or a combination of both.

e. The system selected must be adapted to the sampling rate of the appropriate particle size. Hence, a sequential system cannot be used for Class A. If using sequential systems, particle losses because of the length of the tubes and kinks in the tubing should be considered.

f. Selection of the monitoring system should also involve consideration of the risks generated by sampling during processing.

g. For monitoring purposes, the sample size collected using an automated system will usually be a function of the speed of probing air through the device. It is not necessary to download the same sample volume as that used for formal classification of clean rooms and clean-air devices.

h. 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.

i. Monitoring of particles ≥5.0 µm in Grade A and B zones is particularly important as a diagnostic tool for early detection of failure. Occasional counting of single particles can occur as a result of external interference. However, frequent and continuous high particle counts should raise concerns because it may indicate the possibility of pollution, problems with a heating, ventilating, and air conditioning (HVAC) system, or incorrect practices during routine operations.

j. The particle limits given in Table 7.3 for the at-rest state should be achieved after a short 'clean up' period of 15–20 min in an unmanned state after completion of operations.

k. Monitoring of Grade C and D areas should be undertaken during operation and in accordance with the principles of quality risk management. Warning and alarm limits are determined by the nature of processing. For example, bone cutting may generate numerous particles, and corneal lamellar cutting may generate numerous aerosols, but the recommended at rest state after a clean-up period should be attained.

l. The frequency of particle (viable and non-viable) monitoring should be based on a series of robust data, which demonstrates the normal variability for these parameters in the facility during typical operations. The greater the variability, the more frequent the monitoring should be.

m. For Class A, the monitoring of particles should be carried out during the full duration of critical processing (if tissues or cells are processed according to GMP) or at least every 6 months (for aseptic processing) and at least every 12 months for Classes B and less. Sampling is not required in areas in which particles are generated during the process, which can damage the particle counter, or cause harm (e.g. if bone is processed aseptically). In such cases, air is monitored only before the beginning of the processing.

n. Temperature, relative humidity and differential pressure of clean areas should be monitored every day.

7.4.6.3. *Requalification of clean rooms and laminar flow hoods*

Requalification of clean rooms and laminar hoods is required to support and verify the operating parameters and limits for the critical parameters.

a. Tests to be carried out for the clean rooms should include at least:

i. Air flow and air flow changes per hour according to specifications must be checked (every 12 months);

ii. Absolute integrity of filters: the grade of sealing of the filters and absence of leaks in the filter material will be checked (at least every 24 months or if the resistance across the filer changes abnormally);

iii. Particle counting: the total count of airborne particles (viable or not) will be checked according to specifications;

iv. Temperature/relative humidity will be recorded during the test and will be checked according to specifications (every 12 months);

v. Differential pressure between different areas will be checked according to specifications (every 12 months);

vi. Recovery test (normally tested for clean rooms classified as Grade A and B): the time required for a clean room to recover after a particle-generation event (at least every 12 months).

b. Tests to be carried out for the laminar flow hoods should include at least:

i. Speed and uniformity of the air (every 12 months): the average speed must meet the specified acceptance criteria;

ii. Absolute integrity of filters (every 12 months): the grade of sealing of the filters and the absence of leaks in the filter material will be checked;

iii. Particle counting (at least every 6 months): the total count of airborne particles (viable or not) will be checked according to specifications;

iv. Electronic test (every 12 months): all the operating controls will be checked (light, ultraviolet light, fan) and alarms;

v. Smoke test or Ki Discus test (for biological safety cabinets) (every 12 months). The objective is to study the air flow inside and outside the cabin with the help of a smoke generator.

All these tests should be undertaken by qualified professionals at least in an at-rest situation. In both cases, the particle counting test should be done also in an in-operation situation. Less frequent requalification may be acceptable based on data from self-contained facility monitoring systems that continuously measure particles, temperatures, humidity and differential pressure.

7.4.7. Environmental microbiological monitoring

Microbiological monitoring of clean rooms and laminar flow hoods is mandatory and should be done in accordance with:

a. EN ISO14698 *Cleanrooms and associated controlled environments – Biocontamination control* [2]; and

b. EU GMP Annex 1.

Microbiological monitoring is required during processing, during processing validations, and to demonstrate if cleaning and sanitation methods are adequate (routine cleaning and cleaning after maintenance breaks). Different sampling methods, reagents and incubation methods are described in Chapter 8.

Microbiological monitoring done during the processing of tissues and cells (with settle plates, volumetric air sampling, contact plates, swabs) should provide information about the quality of the environment during processing and allow tracking of trends in the clean room. Surfaces (with contact plates and swabs) and personnel (with glove prints or fingerprints [FIPS]) should be monitored after critical operations. The frequency of sampling should take into account the processes and activities of the staff. At least for aseptic processing (in Grade A/B clean rooms), monitoring must be frequent during the entire processing period. Sampling methods used in operation should not interfere with zone protection. Results of monitoring should be considered when making the decision whether tissues or cells can be released.

Results of monitoring should be considered when making the decision whether tissues or cells can be released.

A sampling plan may be developed to evaluate the impact of the personnel on the environment, the effect of the environment on the product and the effectiveness of cleaning and disinfecting procedures.

To define and control microbiological hazards it is required to identify the potential risks of the processing steps and of the tissues or cells themselves, as well as the probability of these risks and corrective actions to minimise the risks.

Tissue establishments must have a monitoring programme that specifies:

a. acceptance limits of microbial contamination (action level, alert level);

b. sampling plan and frequency;

c. sampling methods and equipment (see Chapter 8);

d. sampling culture media and incubation of samples (see Chapter 8);

e. analyses and evaluation of results (including trend analyses);

f. handling of out-of-specification results.

Recommended limits for microbiological monitoring of clean areas during operation are shown in Table 7.4. Alert and action levels for microbial contamination should be determined and consequent counter-measures specified for the event if these levels are exceeded. 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 routinely and, if necessary, changed due to the variability of processes. The alert level emphasises an acceptable number of microbial contaminations, the excess of which is a warning against the risk of irregularities. 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 level lower than the limits of intervention (typically 50% of the allowable limit). The action level emphasises a certain level of microbial contamination that necessitates immediate corrective action and corrective

measures. The value of the intervention limit is typically 80% of the allowable limit. In Grade A and B areas, detected colonies must be identified to the genus or species, and for other cleanliness Grades according to the microbial-monitoring programme of the tissue establishment. Figure 7.1 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 non-conformity and should be identified.

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

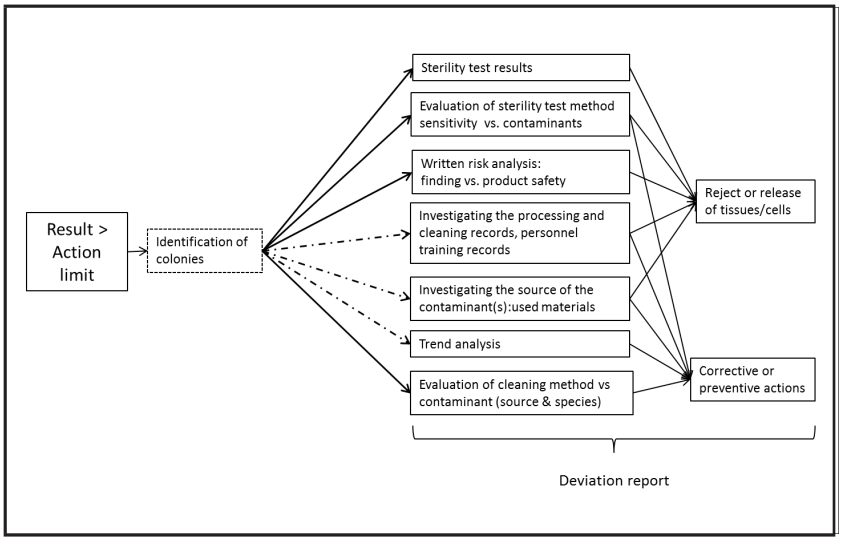
Table 7.4. Recommended limits for microbial monitoring by EU GMP Annex 1 Manufacturing of Sterile Medicinal products (see GMP Annex 1 and EU Guidelines for Good Manufacturing Practices)

Grade	Recommended limits for microbial contamination [a]			
	Air sample (cfu/m³)	*Settle plates, diam. 90mm (cfu/4 hours)* [b]	*Contact plates, diam. 55mm (cfu/plate)*	*Glove print, 5 fingers (cfu/glove)*
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

a These are average values.
b Individual settle plates may be exposed for less than 4 hours in which case the limits should be appropriately reduced.

Figure 7.1. Decision tree on topics and actions to be considered if microbiological-monitoring results (number of colonies) exceed the action limit

In Grade A and B areas, detected colonies must be identified to the genus or species, and for other cleanliness according to the microbial-monitoring programme. Solid lines describe minimal action to be considered, and dashed lines describe topics of broader investigations.



7.4.8. Cleaning

Appropriate sanitation of clean areas is of utmost importance to achieve environmental requirements. Cleaning should be done according to a schedule and procedure that has been validated to achieve the required level of cleanliness. All cleaning procedures should be documented. Cleaning should be done by personnel trained for the procedure, clean-room environment, workflows and gowning. More than one cleaning agent should be employed (rotation of disinfectants) to prevent formation of resistant bacterial strains, and to have wider microbial activity. Cleaning products could include antibacterial, fungicidal, virucidal and sporicidal products. They are made

up of broad-spectrum disinfectants containing quarternary ammonium compounds, stabilised chlorine dioxide, hydrogen peroxide and sodium hypochlorite. There is a risk that microbes could develop resistance so products are rotated to reduce that risk. Disinfectants and detergents used in Grade A and B areas should be sterile before use. Monitoring of the clean room should be undertaken regularly to detect development of resistant strains. Fumigation may be useful for reducing microbiological contamination in inaccessible surfaces.

Some tissue banks and cell banks accept material for autologous use from donors infected with the HIV, HBV or HCV. In such cases, separate processing runs should be done and validated cleaning procedures applied. Forceps to pick up sharps should be used before discarding in a sharps container. If blood or body fluids are present on the surface, they should be cleaned with disposable absorbent material and discarded in containers for biohazardous waste. Afterwards, 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 (usually 1:100 dilution) and the surface allowed to dry.

Inactivation of prions should be considered if risk of prion contamination has occurred (e.g. tissues or cells from a Creutzfeldt–Jakob disease (CJD)-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 [3].

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

7.4.9. 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.

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 clean rooms.

Only the minimum number of personnel required for efficient processing should enter 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.

Required clothing should be chosen based on the process and grade of the working area. Minimum requirements modified from EU GMP Annex 1 are listed in Table 7.5. Outdoor clothing should not be brought into changing rooms that lead to Grade B and C rooms. 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 be provided at each work session (or at least once a

day if monitoring results justify it). Gloves should be disinfected regularly during operations. Masks and gloves should be changed at least at each working session.

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.

Equipment and instruments should be of quality appropriate to their intended functions. Requirements for equipment have been described more in detail in Chapter 2. In order to avoid cross-contamination, the tissues from one donor should not come into contact, at any time during processing or during storage, with tissues from another donor. A separate set of clean, sterile instruments should be used for each donor. Furthermore, tissues that must be processed further (e.g. lyophilisation, sterilisation) should be treated as a single donation. Each tissue should have a batch number that is also recorded in the processing records.

Table 7.5. Minimum clothing requirements (content has been modified from EU GMP Annex 1 Manufacture of Sterile Medicinal Products)

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	Appropriate clean shoes or overshoes
	Gloves	Dependent upon the process
Grade C	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
	Shoes	Appropriate clean shoes or overshoes
	Gloves	Sterile, non-powdered rubber or plastic gloves
Grade A/B In general, the protective clothing material should shed no fibres, and clothing should retain the particles shed by the body	Facemask	Sterile, single use. Eye protection/coverage is dependent upon the process
	Cap	Headgear should totally cover hair, beards and moustaches; it should be tucked into the neck of the suit
	Suit	Sterile coverall
	Shoes	Sterilised or disinfected footwear, boot-like structure to enable the trouser-legs to be tucked inside the footwear
	Gloves	Sterile, non-powdered rubber or plastic gloves

7.5. Quality control

7.5.1. General

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. Evaluation of the quality controls undertaken on the tissues and cells should be established and each should be described in written procedures. The written procedures should at least include the test method, the sample size and the accepted criteria. The minimum requirements for evaluation for each type of tissue and cell are described in tissue- and cell-specific chapters (see Chapters 16–26). The results of all tests or procedures should become part of the permanent 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 protocol itself.

7.5.2. Microbiological control

In many cases, it is not possible to exclude contaminated material during processing because the tissue originates from naturally contaminated parts of the body, and disinfection is not 100% effective. The microbiological safety of tissues and cells is based on donor selection and minimisation of initial contamination, control and monitoring of contamination during the entire transplantation process, and validated methods of decontamination or inactivation during processing of tissues and cells, or sterilisation of non-viable tissues.

Chapter 8 describes microbiological assessment of the starting or incoming tissues and cells, in- process controls, and the final product. Microbiological controls must be done on starting or incoming tissues. In-process testing should be undertaken at relevant processing steps; for instance, after a stage of decontamination or inactivation, or after washing or a change in storage solution. Whenever possible, a tissue or cell sample of the final product should be tested. In addition (or, in exceptional cases such as an exclusive sample), spent storage or

culturing medium or final washing solutions can be used for testing with sample filtration. Every effort should be made to take representative samples. It is widely recognised that swabbing is not an effective method of tissue sampling and that small tissue samples are frequently not representative. Chapter 8 also describes methods of microbiological controls. Sampling and testing methods must be validated to show the representativeness of the sample and the suitability of the selected methods.

Various procedures exist for securing microbiological control, such as decontamination by antibiotics, or physicochemical methods. Some standard sterilisation procedures, such as heat sterilisation or sterile filtration, are not applicable for preparations of tissues and cells. If physicochemical methods are intended to be applied, these procedures must be adapted to the type of tissue or cells 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. Some micro-organisms may survive the antibiotic treatment, but are not detected by microbiological testing due to sampling error, and can recover when conditions change. The terminal sterilisation designation applies when the product, in its final package, is submitted to a chosen sterilisation procedure resulting in a sterility assurance level (SAL) of 10^{-6}.

Non-conforming products must be identified and separated from compliant products. The fate of non-compliant products will be decided by the RP in charge of the tissue establishment.

7.6. Packaging and labelling

Packaging and labelling are described in more detail in Chapter 12.

7.7. Storage

7.7.1. Methods of storage

Tissues and cells should be stored according to currently accepted best practice, based on the best available scientific evidence and according

to GMP, as appropriate, for tissues and cells. All procedures associated with storage of tissues and cells must be documented in SOP.

Storage areas must be kept clean.

Currently used methods of tissue and cell storage include:

a. storage in organ culture media above room temperature (e.g. 31 °C for corneas);

b. hypothermic storage (refrigeration) between 2–8 °C;

c. storage at ambient temperature (for freeze-dried products);

d. freezing with or without the use of cryoprotectants.

Freezing can disrupt tissues and cells. Hence, the method of freezing used must take into account the eventual use of tissues and cells. If cell viability must be maintained, penetrating cryoprotectants such as dimethyl sulfoxide (DMSO), 1,3-propanedial (PROH) or glycerol must be added before tissues or cells are frozen. In such cases, the rate of cooling must be controlled to prevent formation of ice crystals within cells, which can result in damage and loss of tissue integrity. Once cryoprotectants are added, cells are placed in a controlled-rate freezer that gradually reduces the temperature of the mixture in accordance with a given protocol. The rate of cooling should take into account the volume of the cells being cooled. Post-thaw viability of cells should be assessed to determine the effectiveness of the cryopreservation and storage method used.

In the case of multilayered tissues, diffusion of the cryoprotectant into the tissue and the effectiveness of control of freezing will depend on whether the tissue consists primarily of the extracellular matrix or dense cellular layers. Loss of tissue integrity can occur in certain regions (e.g. inner layers) if the cryopreservative is distributed unevenly. Hence, the size and type of tissue must be taken into consideration when validating the method used for cryopreservation.

The rate of cooling for each tissue and cell preparation should be monitored and recorded to ensure that any deviations from the validated protocol are noted. If post-thaw viability and structural integrity is

not compromised, tissues may be cryopreserved by placing them in a freezer without addition of cryoprotectants.

7.7.2. 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 selected maximum storage period should be based on data from published studies, stability testing by the establishment, or 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), regulations).

When relevant for the type of tissue or cells, the time of procurement should also be indicated.

In certain specific cases, it could be possible to prolong the expiry date, especially in the case of reproductive tissues and cells for partner donation or in cases of fertility preservation (see Chapters 25 and 26).

7.7.3. Risk assessment

A documented risk assessment approved by the RP must be undertaken to 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. Remote alarms and auto dialler systems must be checked regularly.

7.7.4. Storage temperature

Refrigeration devices/incubators containing tissues and cells should be suitable for the use intended, and 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, should be employed on all incubators, storage refrigerators, freezers and liquid nitrogen tanks (see Chapter 2).

Temperature ranges for storage of tissues and cells [4] are shown in Table 7.6.

Table 7.6. Temperature range for storage of tissues and cells

Storage condition	Temperature limits (°C)
Vapour phase or liquid nitrogen	<–135
Deep frozen [a]	–80 to –60
Frozen [b]	<–15
Refrigerator [b]	2–8
Cold or cool [b]	8–15
Room temperature [b]	15–25

a Based on general practice.
b Based on the *European Pharmacopoeia*.

7.7.5. Cross-contamination during storage

Every effort should be made to avoid cross-contamination of material stored in vessels containing liquid nitrogen. Frozen tissues must be double-wrapped during storage. This is crucially important for storage with liquid nitrogen owing to the accumulation of microbial contaminants in liquid nitrogen storage vessels. The seals and the material employed must be validated for their use at the designated storage

temperature and the conditions of use to demonstrate that the packaging and labelling can retain their integrity under such conditions.

7.7.6. Quarantine

All human tissues and cells that are stored before determination of their suitability must be kept under quarantine. Quarantined tissues 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 product.

7.8. Release

7.8.1. Release procedure

Release is the act of certifying compliance of a specific tissue or batch of tissues with the requirements and specifications. Before any tissue is 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 the RP according to the relevant local SOP. There must be a SOP that details the 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 transplantation and implantation, and will include:

a. approval of donor eligibility by the RP or designated person;

b. review and approval of the processing and storage record (including environmental-monitoring records);

c. final inspection of the label and container to ensure accuracy and integrity;

d. results of screening tests on incoming material and in-process testing;

e. specifications for final product release based on testing results used to determine final release (e.g. microbiology test results; if necessary and justified, release of the final product can be undertaken on a 'negative-to-date' basis).

The items indicated in the processing and storage record should contain at least:

a. the procurement file and/or the release statement of the person responsible for procurement;

b. type of tissues and cells processed and/or stored;

c. quantitative and qualitative description of tissues and cells processed, preserved and/or stored;

d. date and time of each stage of processing and storage, the identification of persons responsible for each step and the identifying reagents and materials used (batch number and expiry date);

e. status of tissues and cells at all stages of processing and storage (i.e. quarantine, released for therapeutic use, release for manufacturing of medicinal products, *in vitro* research, etc.);

f. use of antibiotics, antibiotic composition and incubation period (if applicable);

g. type and amount of reagents used;

h. procedures and records concerning the processing of tissues and cells (if applicable);

i. processing data (preparation, culture technique, incubation, treatment chemicals);

j. data on techniques of decontamination, sterilisation or viral inactivation;

k. results of specific quality testing, depending on tissue and cell type (e.g. human leucocyte antigen (HLA), histology, radiology results, tissue or cell viability, number of CD34 cells, etc.);

l. procedures and records concerning the preservation of tissues and cells (e.g. cryopreservation, trace of the cooling curve, glycerolisation, lyophilisation), if applicable;

m. date and time of storage;

n. method of storage;

o. storage temperature;

p. expiry date;

q. identification of tissues and cells (i.e. donor identification code plus product code).

Access to registers and data must be restricted to authorised persons. These records must be kept for a minimum of 30 years after clinical use or discard of tissues and cells.

The person responsible for the release of tissues or cells should sign a statement that specifies fulfilment of all ethical, legal requirements and quality release criteria, thereby releasing the tissues and cells for storage in an inventory of tissues and cells that are available for transplantation, human application or research/educational use.

Released tissues should be physically separated and visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from quarantined tissues.

7.8.2. Exceptional release

In exceptional circumstances, an organisation responsible for human application of tissues and cells (ORHA) may agree with a tissue establishment that tissues or cells that do not meet the normal criteria for release should be released and used in a specific patient on the basis of a risk–benefit analysis taking into consideration the alternative options for the patient and consequences of not providing the tissues. Risk assessment should be documented before acceptance of the exceptionally released tissue. The physician in charge of the recipient patient should, together with the Medical Director and the RP of the tissue establishment, conduct the risk assessment and

the risk–benefit analysis for the particular recipient. These discussions and conclusions should be documented. The treating physician should, in writing, confirm his/her agreement with this exceptional departure from normal procedures. The patient should participate or be informed of the decision-making process and conclusions before providing consent (SOHO V&S: *Guidelines for healthcare professionals on vigilance and surveillance of human tissues and cells*). Exceptional release may not preclude follow-up testing, donor screening or other quality-assurance measures described in the SOP.

7.8.3. Disposal of human tissues and cells

There must be a documented policy for discarding of tissues and cells that are unsuitable for clinical use. Records should include details of date and methods of disposal and reasons for discarding the material. The material should be appropriately handled 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 tissue establishment'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 for the human body.

7.9. References

1. ISO Technical Committee 209 (ISO/TC 209). ISO 14644-1, Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness.
2. ISO Technical Committee 209 (ISO/TC 209) EN ISO 14698-1 Cleanrooms and associated controlled environments – Biocontamination control.
3. Taylor DM. Inactivation of prions by physical and chemical means. *J Hosp Infect* 1999; **43** (Suppl): S69–S76.
4. European Directorate for the Quality of Medicines & HealthCare. European Pharmacopoeia, General notices, general

chapter 1. Ph. Eur. 8th Edition. Strasbourg, France: Council of Europe; 2013.

Related document: Appendix 11 – Example of a process validation

Chapter 8

Principles of microbiological testing

8.1. Introduction

This chapter addresses the control and examination of microbiological and endotoxin contamination of human tissues and cells, which is critical for ensuring the quality and safety of human tissues and cells 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. Guidance on microbiological monitoring for particular tissue and cell processes is provided in relevant tissue- and cell-specific chapters. However, this chapter defines the general principles that should be adopted in developing a comprehensive strategy for microbiological testing. The testing methods to be applied in the laboratory are described and, most importantly, principles regarding validation of those methods are defined.

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

8.2. Microbiological examination of donors: blood cultures

Microbiological examination of potential donor blood (blood cultures) can be a very useful tool to help in the diagnosis of bacteraemia and other infections in donors of tissues and cells. It is generally agreed that for a blood culture to have sensitivity and specificity as well as predictive values of microbial contamination, it is important to follow a clear protocol that addresses sampling (central or peripheral line), the method used for skin disinfection, the amount of blood obtained, the number of blood cultures for aerobic and anaerobic (at least two for each is recommended) and the need to maintain cultures for ≥5 days. 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.

Tissues can be procured from deceased donors, either brain-dead donors (heart-beating donors) or donors after circulatory death (non-heart-beating donors). Blood cultures can be obtained if the heart is still beating. The results of these blood cultures provide useful information about the clinical status of the donor (e.g. infections with objectionable organisms) and reduce the potential of erroneous results due to agonal spread and *post mortem* bacterial translocation from blood taken from a non-heart-beating donor. Nevertheless, information about *post mortem* bacteraemia and fungaemia at the time of procurement may also be useful to assess the quality and suitability of tissues and cells. In non-heart-beating donors of tissues and cells, blood cultures may be obtained some time after cardio-circulatory arrest, and the results can be influenced by agonal spread and *post mortem* bacterial translocation. In this situation (in which organs and tissues 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 conditions under which the deceased donor are stored may be highly variable.

In the case of positive blood cultures, published data on the microbiological examination of bone surfaces by swab cultures show little correlation between the two types of culture. In very few cases, the bacteria or fungi found in blood cultures are identical to those found in procured bones.

The procedure for microbiological examination of the donor using blood cultures should consider the specific clinical situations of the donor, such as the use of antibiotic treatment in the previous 72 h.

Results of blood cultures should be interpreted in accordance with risk-assessment analyses of the donor taking into account the blood sampling method, if the sample was obtained when the donor was in cardio-circulatory arrest, if the donor received antibiotic or anti-thrombotic therapy, the time elapsed between cardiac arrest and collection of blood samples, the time elapsed between cardio-circulatory arrest and refrigeration of the body, and how the tissues and cells were procured.

In heart-beating donors, blood cultures can be a useful supplementary tool for evaluation of donor suitability. Whether blood cultures are valuable for evaluation of the quality and safety of specific tissues and cells (especially in donors after circulatory death) and if the tissues and cells are not terminally sterilised, is dependent upon numerous factors, and should be determined based on knowledge of the particular situation [1–7].

8.3. General considerations for microbiological control of human tissues and cells

The approaches outlined in this section cover the microbiological control of procurement, processing, storage and release of tissues and cells. The approaches cover the minimum standards to control the microbiological safety of preparations of human tissues and cells such as heart valves, blood vessels, bone preparations, skin, amniotic membranes, corneal tissues or products of haematopoietic stem cells. Deviations from such standards should be justified and the suitability of the intended test method must be demonstrated. Factors such as

samples containing antibiotics or very small sample amounts may affect the sensitivity of tests leading, in the worst-case scenario, to false-negative results.

8.3.1. **Microbiological concepts for the detection of bacteria and fungi**

8.3.1.1. *Microbiological examination of procured tissues and cells*

8.3.1.1.1. *Sampling*

In principle, microbiological examination must be carried out on a sample of the procured tissues and cells. In addition, where applicable a sample of the storage, transport or rinsing solution should be tested. In cases where the nature of the procured tissues and cells does not allow sampling of the starting material, an alternative sampling approach may be undertaken (e.g. surrogate testing on liquids and/or cells last in contact with the starting material).

8.3.1.1.2. *Testing*

Considering the nature of the tissues and cells and any subsequent processing steps, either testing for sterility, absence of specific micro-organisms or microbial enumeration must be carried out. The approach should follow the procedures outlined in Chapters 2.6.1, 2.6.27, 2.6.12 or 2.6.13 of the *European Pharmacopoeia* (*Ph. Eur.*). For each procedure, aerobic, anaerobic and fungal testing under appropriate incubation conditions must be conducted. Testing should be extended to specific micro-organisms, which may not be detectable with common culturing media and, if indicated, additional tests for specific infectious agents, such as mycoplasma or mycobacteria, should be undertaken. If applicable, an exclusion list for non-acceptable micro-organisms should be compiled.

8.3.1.2. *In-process testing*

A risk assessment for the microbiological contamination should be carried out depending on the nature of the tissues and cells, the origin, procurement, critical steps during processing, and their in-

tended application. Critical steps during processing are steps in which exposure to micro-organisms and contaminations of the tissues and cells may occur (e.g. during manual processing of tissue preparations). In particular, the risk of microbiological contamination should be assessed when using band saws, bone mills, and cutting tissues to size.

In-process testing should be done at relevant steps of the production process. Generic criteria for undertaking in-process testing are:

a. testing for any type of contamination before a decontamination procedure (e.g. antibiotic soaking) which has not been validated to remove all contaminants; in this case, a list of contaminants (usually pathogens and micro-organisms) that are not guaranteed to be removed by the decontamination procedure should be defined;

b. after a stage of decontamination or inactivation, before final storage;

c. at washing or changing of the storage medium, particularly when decontamination processes cannot be applied.

For in-process testing, storage or transport solution, used tissue or cell culture medium or other suitable samples may be tested.

8.3.1.3. *Microbiological examination of the final product or preparation*

8.3.1.3.1. *Sampling*

After completion of all processing steps, samples of the final product – if possible – and tissue or cell culture media, storage or preservative solutions – if applicable – must be tested.

In exceptional cases, if sampling of the final product is not feasible, used tissue or cell culture medium storage, rinsing or washing solution can be used instead for microbiological examination of the final product.

If an appropriately validated terminal sterilisation process is applied, testing of samples within a batch has to be undertaken to monitor the effectiveness of the terminal sterilisation process.

8.3.1.3.2. *Testing*

Microbiological control of the final product should be done in accordance with *Ph. Eur.* 2.6.1. Especially for cell suspensions, other liquids and injectable materials as well as automated culture systems can be used (*Ph. Eur.* 2.6.27). Many types of bacteria and fungi found in environmental and clinical settings are detectable by such test methods. If there is a need to extend the testing to micro-organisms with specific culture conditions, suitable culturing methods should be applied. If release of the product is necessary before the end of the officially verified/required incubation period, 'negative-to-date' reading of the results may be carried out. As an alternative, rapid microbiological methods should be considered, especially for preparations of tissues and cells with a short shelf-life. Independent of the applied method, their suitability must be shown with respect to specificity, sensitivity and robustness. Deviations from these standards should be justified.

In general, suitable microbiological tests must be applied for each batch of a final preparation of tissues or cells after final packaging. If this is not applicable, sampling must be completed immediately before final packaging or as late as possible during the manufacturing process.

8.3.2. **Testing for micro-organisms with specific growth requirements**

Depending on the type of preparation and manufacturing 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) or mycobacteria.

Possible sources for mycoplasma contamination include the cellular starting material itself or its procurement, animal- or human-derived raw materials such as untreated sera, personnel in the clinic or manufacturing site, and the entire manufacturing process.

Mycoplasmas can penetrate sterilising-grade filter membranes with a nominal pore size of ≤0.2 µm. They also lack a rigid cell wall, which makes them unsusceptible to antimicrobial agents that target the cell wall. Most other broad-spectrum antibiotics inhibit only the multiplication of mycoplasma but do not kill them, so elimination with antibiotics is virtually impossible.

Contamination with mycoplasma represents a risk for the patient because of transfer of infectious microbial agents into a potentially immunocompromised patient. However, the effect of mycoplasma contamination on the tissue or cell preparation might also be critical. Mycoplasmas are known to alter cell function, leading to changed expression of genes and proteins and cell activity. These alterations can even result in malignant transformation of cells.

Mycoplasma testing should be carried out for cell-therapy products. Risk assessment should be the basis for the decision as to whether mycoplasma testing is necessary for all other tissue- and cell-based preparations. Depending on the type of preparation and its origin, the route of application and the possible impact on the health of the recipient, it may be recommended to test for mycoplasma. Testing should be conducted at manufacturing steps at which mycoplasma contaminations would most likely be detected, such as after pooling or collection but before washing steps. Mycoplasmas are cell-associated micro-organisms that may even locate within the cell, so testing should always include the cellular matrix, if possible.

8.3.3. Testing for bacterial endotoxins

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

The presence of endotoxins in products can result in responses ranging from fever to irreversible and fatal septic shock. Endotoxins are major components of the outer membranes of most Gram-negative bacteria. If bacteria are growing actively they shed small amounts of endotoxins (and large amounts if they die).

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

a. glassware and plastic ware used in the laboratory;

b. washing solutions or water used to prepare media and solutions;

c. media and sera used during cell culture;

d. any components and additives.

Hence, it is recommended to use raw materials certified to be free of endotoxins by their manufacturers.

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

Endotoxins can be detected using the specific limulus amoebocyte lysate (LAL) bacterial endotoxin test (BET). Because of the great 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 products is based on the route of administration (intravenous or intrathecal), the threshold pyrogenic dose, and the volume of the injected product. For certain cellular products 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 BET quantifies only the amount of endotoxin, not the biological impact.

Cell-based assays such as the monocyte activation test (*Ph. Eur.* 2.6.30) might be more suitable for estimating the biological effects [8–10].

8.3.4. Explanatory notes – microbial controls required for specific processing methods

8.3.4.1. *Processing using closed systems*

For specific types of products such as haematopoietic stem cells and similar preparations in which a closed system is used for processing and where no further cell-cultivation steps are conducted, repeated testing steps are not suitable and not yield more information on the microbial status of the product. In such cases, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point may be applicable.

8.3.4.2. *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 (steam, dry heat, ionising radiation, gas). 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.

Procedures and precautions employed for sterilisation are such as to give a sterility assurance level (SAL) of 10^{-6} or better.

If the release of products sterilised in their final container is intended to rely on process data only and not on final product 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. Authorisation by the Health Authority is needed for such an approach.

8.3.4.3. *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 antimycotic 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 changeover in a specific state without growth (which is reversible in an optimal host), microbial cells can escape this treatment. Knowledge about the acceptable quantitative and qualitative microbial load of the starting material before antimicrobial treatment is necessary. Efficacy studies focusing on the usually expected initial state of tissues or cells as well as the type and concentration of antimicrobial agents should be carried out. Based on the outcome of those studies, only temporary treatment and exclusion of specific contaminants in the incoming material should be carried out. Further processing after the decontamination step should be conducted without antimicrobial agents. Methods for final product testing must be evaluated carefully with respect to possible inhibition of microbial growth due to decontaminating agents or their residues. The necessity of endotoxin testing should be evaluated.

8.3.4.4. *Open processing without terminal sterilisation*

Most tissues and cells, including preparations which have been decontaminated, are processed using open processing methods. Open processing is any processing step in which tissues and cells are exposed to the environment at any stage between procurement and packaging. This section relates to open processing methods, where the preparation is not subjected to terminal sterilisation. In general, any risk of contamination from the environment or consumables used during open processing must be reduced. The requirements for microbiological sampling and testing are expected to be most stringent in these situations, and aseptic conditions must be maintained during procurement, transportation and the whole manufacturing process.

If open processing takes place without terminal sterilisation, sampling and microbiological assessment should include the starting material, transport solution, and solutions used to wash tissues and cells. *Ph. Eur.* 2.6.1. provides a means of verifying that the tissues and cells are sterile and where, because of the nature of the tissue or cell-based preparation, the approaches in *Ph. Eur.* 2.6.27 may be applied. If a preparation is not required to be sterile or cannot be rendered sterile, *Ph. Eur.* 2.6.12 and 2.613 describe tests that allow quantitative enumeration of micro-organisms. Such testing may require use of validated methods employing special media and/or conditions to enable growth of such micro-organisms and their detection. In addition, the final product should be tested to ensure quality and safety for clinical use.

8.4. Microbiological testing

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

8.4.1. Sterility testing of solutions or tissue samples pursuant to *Ph. Eur.* 2.6.1

Conditions for sterility testing are detailed in Table 8.1.

Precautions against microbial contamination during a test should be taken (*Ph. Eur.* 2.6.1). At least sub-cultivation should be carried out in a class-A laminar airflow cabinet properly disinfected before the test and no other activity should be held at the same time.

Table 8.1. Incubation conditions

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

The preferred microbiological control procedure is dependent upon the sample material.

8.4.1.1. *Membrane filtration method*

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

8.4.1.2. *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 microbial growth by turbidity. 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 opaqueness 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. Alternative incubation conditions are shown in Table 8.2.

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

8.4.2.1. *Incubation conditions*

Use of an automated culture system may be advantageous, especially for liquid sample material, which already results in turbidity of the culture media immediately after inoculation. Different to *Ph. Eur.* 2.6.1, automated culture systems are mostly preset to an incubation temperature of 35–37 °C, which is based, for example, on the testing of blood and blood products.

Incubation in automated culture systems should, ideally, be carried out over 14 days, especially if risk assessment identifies potentially slow-growing micro-organisms. The incubation time can be adapted to specific requirements arising from the characteristics of the preparation.

The time and temperature of incubation may be too limited to sufficiently account for a broad range of contaminating micro-organisms found in the environment or in tissues and cells. Hence, the incubation conditions detailed in Table 8.2 are recommended as alternatives on the basis of risk assessment, taking into account the expected microbial flora and environmental conditions.

Table 8.2. Alternative incubation conditions [a]

	Aerobic incubation	Anaerobic incubation
Option 1	20–25 °C (automated system), if necessary 30–35 °C (automated system).	30–35 °C (automated system)
Option 2	35–37 °C (automated system), 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 Incubation period is 7 days with an automated growth-based method and 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.

For preparations of tissues and cells with a short shelf-life, the preparations may be released based on an intermediate readout of the test before the incubation period is completed. In the case of a positive readout during the incubation period after release of the preparation, identification of the microbial species and a resistogram must be carried out and the information forwarded to the physician.

8.4.2.2. *Sample volume*

For automated culture systems, sample volumes of ≤10 ml can be inoculated per culture bottle. Very small sample amounts may bear the risk of an enhanced sampling error, so a large amount of sample should be envisaged for inoculation (if applicable and appropriately validated).

8.4.2.3. *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 (FOS).

8.4.2.4. *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 in automated culturing systems) is highly variable. Culture bottles containing adsorbing substances are established for the testing of patient's 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.

8.4.2.5. *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 period of 12 h 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.

8.5. Notes on the validation of microbiological methods for the testing of preparations of tissues and cells

8.5.1. Growth promotion test

Each batch of the microbiological culture medium to be used must be tested for its growth-promoting capacities ('Growth promotion test' in accordance with *Ph. Eur.* 2.6.1 and 2.6.27). A growth-promotion test must not be carried out by the applicant if quality certificates from the manufacturer of the culture media are available that confirm compliance of the media with the requirements of *Ph. Eur.* (batch release documents), and if transport and storage conditions can be guaranteed to not alter the growth promoting capacity of the culture medium. In general, it is recommended to include possible relevant microbial contaminants from the respective tissue preparation or cell

preparation or the environment, for instance, *Propionibacterium acnes* and *Micrococcus* sp., in the assays because of their specific growth properties.

If these certificates are not available, the applicant must demonstrate appropriate growth-promoting capacity. In general, it is recommended to include other possible relevant contaminants from the respective preparation or environment, for instance, *Propionibacterium acnes* and *Micrococcus* sp., in the assays because of their specific growth properties.

Growth-promotion testing should be done for the plates and media used in microbiological monitoring. There should be a formal programme that determines the properties of media for a defined list of organisms. Growth-promotion testing must show that the media are suitable to consistently recover environmental contaminants (assuming that they are present). The standardised list should comprise organisms based on the literature and/or environmental isolates, and should include a reasonable range of 'representative' micro-organisms that could be encountered in manufacturing environments (e.g. Gram-positive rods, Gram-positive cocci, filamentous moulds or yeasts, Gram-negative rods). The list should contain a minimum of five unique microbial strains [11].

8.5.2. Method validation

The method must be validated in the presence of the intended sample material (e.g. transport medium, final product). The basis for method validation is the 'method suitability test' laid down in *Ph. Eur.* 2.6.1 as well as the 'Method validation' laid down in *Ph. Eur.* 2.6.27.

The same conditions must be chosen as for routine testing (e.g. culture conditions, sample type, sample amount). The method suitability test must be undertaken using the bacterial and fungal species indicated in *Ph. Eur.* 2.6.1. *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* as well as the fungi *Candida albicans* and *Aspergillus brasiliensis* should be incubated under aerobic condi-

tions; *Clostridium sporogenes* should be incubated under anaerobic conditions.

It is recommended to complement the microbial spectrum by tissue- or cell-specific micro-organisms such as *Propionibacterium acnes* and *Micrococcus* sp.

For instance, *Propionibacterium acnes* is not readily accessible to skin disinfection due to its prevalence in the sebaceous glands, and detection of this species in tissue preparations is not unusual. *Propionibacterium acnes* grow under anaerobic or microaerophilic conditions as a 'slow-grower' and is associated with particularly long detection time, so it may be included in method validation. If any other micro-organisms are considered to be relevant during processing and if present in the environment, they should also be included in validation studies.

The sensitivity of the chosen method should be shown by inoculating ≤100 colony-forming units (CFU) of the selected micro-organisms in the presence of the product. The microbial count of the dilution used for inoculation must be verified using a suitable method for each assay (e.g. plating on solid media). If a method cannot be used to detect microbial counts of 100 CFU, the limit of detection must be evaluated by experimental studies. Applicability of this method must be assessed in connection with its impact to ensure microbial safety of a product.

Each micro-organism species should be tested in duplicate at least. For evaluation of the robustness of the testing method, it is recommended that assays are repeated in the same way with defined deliberate variations (different staff, batches of consumables, and days).

For comparison, a positive control (without product) must be included in the test for each test strain. For negative control, a suitable amount of sample of the product to be tested must be incubated in the aerobic and anaerobic culture medium, at least in duplicate without inoculating micro-organisms. Positive and negative controls must also be prepared in the event of repeat tests.

Inoculated media must be incubated under the conditions applied in routine testing (temperature, duration) and checked for growth at regular intervals.

Test assays and controls must be evaluated in predetermined intervals during and at the end of the incubation period. Samples for subculture must be taken from positive detected tests as quickly as possible. In the case of microbial growth, the micro-organisms must be identified.

If inhibition of microbial growth by the sample material is identified at validation, the method must be adapted in an appropriate way, for instance, using a higher volume of the culturing media or addition of binding or enzymatic substances that inhibit antimicrobials.

8.5.3. Documentation and interpretation of results

All materials used and working steps undertaken must be documented.

Interpretation of results should include at least the following factors:

a. assessment of the growth of micro-organisms in the presence of the product to be tested and in controls;

b. specification of the microbial count for evaluation of the method;

c. period of time until a positive result has been detected for test assays and positive controls;

d. proof of identity of inoculated micro-organisms.

For negative controls or test assays without detection of micro-organisms, the total incubation period and results of the subculture (including the methods used) must be specified.

8.6. Interpretation of results and actions to be taken

In general, source material that demonstrates contamination must be rejected unless the preparation undergoes decontamination and/or terminal sterilisation, and the detected quantity and quality of micro-organisms can be reliably inactivated or removed by the

intended procedure, or if it is justified by exceptional clinical circumstances (refer to Part B for specific examples).

Contaminated source material should be rejected if processing includes decontamination (but not terminal sterilisation) and risk assessment considering the intended route of administration cannot exclude risk to the recipient even if adequate antimicrobial treatment is initiated. Such source materials should be evaluated on the basis of qualitative (exclusion list for objectionable micro-organisms) and quantitative (microbial count, bioburden) microbiological control tests, and specifications should be given. The decontamination procedure should be shown to be suitable to remove or destroy the type and number of contaminants allowed in the source material. In particular, all multi-resistant micro-organisms (e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum beta lactamases [ESBL]), possible toxin-producing micro-organisms, *Pseudomonas aeruginosa*, *Streptococcus* group A, *Staphylococcus aureus*, *Clostridium* and *Bacillus* sp. (as one particular group) and all yeasts and fungi should be evaluated very carefully, and if appropriate, the tissues and cells should be rejected.

In the case of locally acquired contamination or a local infection, the microbiological result applies only for the tissue where the contamination was detected. If bacteraemia, septicaemia (anamnestic or blood culture) or any other distribution of the objectionable micro-organisms (at procurement, storage, transport, manufacturing) cannot be excluded, other tissues should undergo risk assessment and, if they may be affected (even if not found in samples) and the implantation could harm the recipient, they should also be rejected.

For contaminated autologous preparations or preparations received from a specific heterologous donor, whereby a repeated procurement cannot be conducted or involves a high degree of risk, risk assessment based on the urgency of the application, judgment of infectious risk and treatment options must be conducted. In application of such preparations, measures must include full identification of the contam-

inating micro-organism and their resistograms, as well as adequate prophylaxis of the donor/recipient if the product must be used.

8.7. General considerations for microbiological monitoring of the environment and reagents

Guidance for planning and carrying out microbiological monitoring of cleanrooms, laminar flow hoods and other equipment is described in Chapter 7.

Microbial samples can be taken using four sampling methods: volumetric sampling, settle plates, contact plates, and glove prints (fingerprints [FIPS]). Table 8.3. summarises the characteristics of these sampling methods.

Table 8.3. 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 surrounding the open product. 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. In general, the manufacturer of the sampling instrument will recommend sample sizes, and this recommendation must be taken into account in the design of sampling strategies. Sample sizes ≥1 m^3 should be taken for each measurement. If this sample size results in an unreadable number of colonies, reduced volumes may be employed to monitor Class C and D areas 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.

Method	Air or surface qualitative or quantitative	Notes
Settle plates	Air/qualitative	This is the only method that can provide continuous monitoring of micro-organisms. Can be used to detect bacteria and fungi that descend in a column of air over the plate. Need to determine how long the plates can be open (usually 2–4 h). Exposed plates may be replaced by fresh ones so that the total time of exposure is reached.
Contact plates	Surface/qualitative	Plates or strips can be used. Contact plates can be used to detect contamination by micro-organisms in the immediate vicinity of the work area. The pressure and duration of contact have a significant influence on microbial yield (recommendation: uniform pressure for10 s). Tested surfaces must be cleaned after sampling.
Swabs	Surface/qualitative	Used for wiping of surfaces that cannot be sampled with plates or strips. 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. 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 be transferred onto products. Glove prints (all five fingers) should be taken to assess this possibility. Usually placed on contact plates after processing or before changing gloves. Gloves must not be disinfected immediately before samples are taken. A firm and even pressure should be applied for ≈5–10 s taking care not to damage the agar surface.

8.7.1. Incubation of samples

Environmental-monitoring samples should be incubated at a minimum of two temperatures to detect bacteria and fungi. Incubation for 3–5 days at 20–25 °C followed by incubation at 30–35 °C for an additional 2–3 days has been shown to be sufficient to detect most bacteria and fungi. The method chosen should be validated and standardised very carefully. Alternative methods are acceptable if high recoveries (>90%) of micro-organisms of interest can be demonstrated consistently [11].

If micro-organisms are expected in the environment, and cannot be detected using standard media for environmental monitoring and the temperatures recommended above, the procedure must be adapted accordingly.

8.7.2. Data analyses

Reading of plates and media should be done according to a defined standardised procedure. Identification of colonies should be undertaken according to the environmental monitoring programme of the tissue establishment. According to Good Manufacturing Practice in Grade A and B areas, detected colonies must be identified to the genus or species.

8.8. References

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Chapter 9

Distribution and import/export

9.1. Introduction

This chapter describes the requirements for distribution of tissues and cells and defines recommended controls for their import and export. The term 'distribution' should be understood to mean transportation and delivery of tissues or cells intended for human applications. The entire distribution chain must be validated appropriately, including the equipment used, to ensure the maintenance of critical transport 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 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 distributed by a tissue establishment:

a. to a clinical facility within the same country, where they will be applied (i.e. allocation);

b. to another tissue establishment within the same country for local distribution.

Cross-border movement of tissues and cells includes transfers:

a. to a tissue establishment outside the country (i.e. export);

b. from another country to a clinical facility or tissue establishment in the country (i.e. import).

For the movement 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. However, several EU member states opt to apply more stringent requirements than those in the Directives and to consider this movement in the same way as import/export involving countries outside the EU (referred to as 'third countries'). Please refer to section 9.4.5 for further information.

9.2. 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. 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, an automatic filling mechanism or a standardised manual procedure must be in place to ensure and document that an adequate level of liquid nitrogen is maintained during transport. Temperature data loggers are often placed within the secondary packaging. The data from temperature loggers is downloaded and checked to ensure that the temperature during transport was within acceptable limits. Temperature indicators are sometimes used to indicate exposure to excessive temperatures.

Containers/packages should be secured and labelled appropriately (see Chapter 12).

Written agreements should be in place when transport of tissues and cells is entrusted to transport companies. In EU member states, if the transport is subcontracted, a written agreement must be signed

between the transporter 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 transportation (see also Chapter 2). In any case, transport-related serious adverse events should be identified and reported to the Health Authorities (see Chapter 14)

9.3. Allocation

The allocation of tissues and cells should be guided by clinical criteria and ethical norms. The allocation rules should be equitable, externally justified 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 upon request.

9.3.1. Visual examination

Packaged tissues or cells should be examined visually for appropriate labels, expiry date, container integrity and security and any evidence of contamination prior to being dispatched (see Chapter 12).

9.3.2. Medical competence

Distribution for clinical application should be restricted to hospitals, physicians, dentists or other qualified medical professionals and must comply with any national allocation regulations (see Chapter 10).

9.3.3. Documentation

The place, date and time of pick-up and delivery (including time zone where relevant) and identity of the person receiving the tissues and cells should all be recorded and maintained in the tissue establishment from which the tissues or cells are used.

Any transportation must be accompanied by a transport record that is attached to the package.

9.3.4. Recall and return procedures

An effective recall procedure must be in place in every tissue establishment, including a description of the responsibilities and actions to be taken in the case of a recall. This must include procedures for the notification of the relevant Health Authority(ies).

A documented system must be in place for the handling of returned products, including criteria for their acceptance into the inventory, if applicable.

9.4. Import and export

9.4.1. Underlying principles

Import and export between countries should be done only through legally authorised tissue establishments who have the competence to guarantee a sufficient level of quality and safety evaluation and to meet traceability requirements. They should be specifically authorised for:

a. import and/or export of human tissues and/or cells intended for human application;

b. import and/or export of tissues or cells intended for the manufacture of medicinal products derived from human tissues and/or cells;

c. import of procured human material intended for processing, storage or banking in a tissue establishment or cell establishment in their country.

As a general rule, if clinicians or healthcare facilities identify a need to import tissues or cells, they should organise this through a written agreement with a licensed tissue establishment in their own country. Third-party agreements must specify the terms of the relationship and the relevant responsibilities, as well as the protocols to be followed, to meet the required performance specifications.

9.4.2. Import

Tissue establishments that wish to import tissues or cells should be able to demonstrate that the need cannot be adequately met by comparable material available from sources within their country or that there is another justifiable reason for the import. They should also be able to justify the import in terms of accessibility, quality, speed of supply, risk of infection, quality of service, cost-effectiveness or scientific or research needs. They should ensure that any material intended for import is consistently sourced under the legal and ethical requirements of their country and the exporting country. If the importing tissue establishment cannot satisfy itself that ethical standards are in place in the country of origin, the tissues or cells should not be imported.

The safety and quality characteristics of the tissues or cells to be imported should be equivalent to those in place within the importing country. Imports should be accepted only from countries that have established procedures to authenticate the legitimacy of exporters and the provenance of the donated material they supply. Exporters should be asked to provide evidence of compliance with the regulations that they are required to observe before any orders are placed with them.

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).

Companies that act as distributors, often also carrying out import and export activities, have responsibilities equivalent to those of tissue establishments for ensuring the equivalent safety and quality requirements, for maintaining traceability and for having adequate vigilance systems in place. Fulfilment of these requirements implies having staff and expertise (including medical expertise) to evaluate donor selection criteria and reports of adverse incidents and reactions. In the EU, only tissue establishments authorised for importation from non-EU countries can carry out such activities.

9.4.2.1. *Routine importation*

The importing tissue establishment should assess and document that the exporting tissue establishment complies with the quality and safety recommendations in this Guide. This includes respect for the fundamental ethical principles of consent, non-remunerated donation, anonymity and respect for public health. The evaluation should include at least the following:

a. the general quality and safety systems at the exporting establishment, including organisational chart, staff training, facilities, processing methods, validation studies, traceability and biovigilance systems, licenses and accreditation (including lab certification/authorisation) and donor blood testing;

b. a review of the safety and quality of individual dispatches of tissues or cells (i.e. confirmation of donor consent, confirmation of donor sample testing and their results, donor eligibility records, description of the tissue or cells, transportation arrangements, etc).

Potential language barriers should be considered and a common language agreed upon for all donor and tissues- and cells-related documentation.

A Service Level Agreement or contract between the exporting and importing tissue establishments that clearly defines roles and responsibilities is a basic requirement. Agreed procedures for the transport of the tissues and cells from the country of origin to the tissue establishment in the importing country should form part of the contract and should specify the methods to be followed to ensure the maintenance of the required environmental conditions, of the package integrity and of compliance with agreed timeframes. Such transportation should be direct, without intermediate stops when possible, using an approved courier. The courier or transportation service must maintain records of pick-up and delivery, as well as the container's contents, so that complete traceability is ensured.

The agreement should specify how tissues and cells will be identified. Unique identifying codes should allow traceability and a formal and unambiguous identification of all tissues and cells.

Agreements between importing tissue establishments and suppliers in other countries should include provisions for the performance of audits at the exporting facility and should require that any changes to authorisation status be immediately communicated to the importing tissue establishment.

9.4.2.2. *'One-off' importation*

There may be cases where exceptional or one-off importation is necessary for a single patient. In these cases, the importing tissue establishment should ensure that there exists a documented evaluation of the safety and quality of the tissues or cells being imported. The importing tissue establishment should keep the documentation obtained from the exporting tissue establishment for the time period specified in national regulations (e.g. 30 years in EU member states).

In limited cases (e.g. in emergency situations or for immediate transplantation) the import of certain tissues and cells may be directly authorised by Health Authorities. Health Authorities should take all the necessary measures to ensure that imported tissues and cells respect the national quality and safety standards.

9.4.3. Customs clearance

For clearance of Customs, all tissues and cells supplied from abroad require a clear description of the content of the consignment and its destination and must be labelled as described in Chapter 12. It is important that frozen tissues and cells, which are usually packed in dry ice or stored in liquid nitrogen or in a dry shipper, must not be delayed at border crossings. Therefore, it may be expedient for the importer to inform Customs of a prospective consignment and any enquiries by Customs should be answered promptly. The agreement with the exporter should define responsibilities for meeting the cost of transport, refrigeration and/or storage at a Customs facility for any items that may be detained pending Customs enquiries.

9.4.4. Acceptance at the establishment

Each importing establishment should have a documented procedure and specifications against which each consignment of tissues and cells, together with its associated documentation, is verified for compliance with the written agreement in place with the exporter. Any non-compliance should be reported to the exporter. Consignments should be examined for any evidence of tampering or damage during transport.

Tissues and cells should be stored in quarantine in an appropriate secure location under defined conditions until such time as they, along with their associated documentation, have been verified as conforming to requirements. The acceptance or rejection of received tissues and cells should be undertaken and documented in accordance with the guidance shown in Chapter 10.

9.4.5. EU requirements for importing tissues and cells

In April 2015, a new implementing Directive regarding the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells was adopted. Commission Directive (EU) 2015/566 stipulates that tissues and cells must be imported into the EU by tissue establishments authorised for such imports by competent authorities. It also lays down the obligations of the importing tissue establishments and competent authorities of EU member states who need to verify that imported tissues and cells meet quality and safety standards equivalent to those in place in the EU legislation for tissues and cells. These 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 with 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 authorisa-

tions. 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 new Directive concerns the use of 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.

A limited number of exceptions to certain procedures are foreseen for situations whereby certain tissues and cells are imported on a one-off basis, whereas tissues and cells imported under direct authorisation of the competent authority of EU member states (i.e. in emergency situations or for immediate transplantation) are not affected by the new procedures.

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.

9.4.6. 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.

9.5. International co-operation

For some transplant patients, including sensitised patients, it may be difficult to find a match within their own country. In these cases, co-operation between countries is necessary and in some cases it may be necessary to identify suitable donors worldwide. International co-operation and exchange of tissues and cells is necessary to increase the chances of providing tissues and cells for patients in life-threatening situations. For these reasons, it is important to ensure that there is good co-operation between organisations that allocate internationally. Registries should be in place for all imported and exported tissues and cells to ensure transparency in the process.

Chapter 10

Organisations responsible for human application

10.1. Introduction

Tissues or cells shall not be distributed without an order from a physician or other authorised health professional. Once tissues and cells arrive at clinical application units (hospitals, clinics, doctor surgeries, dental surgeries (offices), assisted reproductive technology [ART] centres) for application in a patient, the responsibility for maintaining the quality-assurance chain is transferred to that organisation. This includes the need to store and handle tissues and cells correctly, inform patients of the risks involved in the use of the material, maintain traceability, and detect, report and investigate adverse outcomes. The European Union (EU)-funded project SOHO V&S (Vigilance and Surveillance of Substances of Human Origin) considered these issues in a working group and developed a guidance document for clinical users. Much of the text in this chapter has been adapted from that guidance document [1].

10.2. Appropriate use

The risk associated with the human application of tissues and cells is, in general, regarded as being very low, particularly if the donated

tissues and cells have been highly processed, blood and other cells have been removed or if it has even been terminally sterilised. However, the application of human substances always carries some risk. Adverse outcomes are rare, but viruses and bacteria have been transmitted by a wide range of tissue and cell types. These events are due to failures in monitoring the quality and safety of tissues provided for clinical use [2].

Physicians must give careful consideration to the risks and benefits of treating a patient with substances of human origin such as tissues or cells, and consider the availability of alternatives before making a decision. Once the physician has decided that the application of tissues or cells is the most appropriate treatment, all health professionals involved in treating the patient should be conscious of their exceptional nature. Tissues and cells are donated altruistically for the benefit of patients in need and are often in short supply. Hence, only the required amount should be ordered and wastage should be avoided.

10.3. Choosing a supplier of tissues or cells

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 human tissues and cells, such as those mentioned above. In most cases, tissues and cells are provided by tissue establishments. In cases of 'direct distribution' procurement organisations, especially collection centres that procure haematopoietic stem cells (HSC) for allogeneic (related and not-related) use, they send a donation directly to transplant centres without any intermediate steps such as processing or storage.

Before requesting tissues or cells from a particular tissue establishment or procurement organisation (e.g. HSC collection centre), the ORHA should confirm that the supplying tissue establishment/procurement organisation are working to appropriate standards of safety and quality. Due to the obligation to maintain donor–recipient anonymity, in the case of collection of HSC for non-related allogeneic use, there is no direct collaboration between a procurement organisa-

tion and transplant centre, and the HSC Donor Registry is responsible for ascertaining whether the collecting centre has fulfilled appropriate safety and quality standards.

If the tissue establishment is within the EU, it should be able to provide an authorisation certificate from the appropriate Health Authority (Competent Authority for Tissues and Cells). This certificate should specify the types of tissues or cells and the general activities for which the establishment is authorised. The establishment should be inspected regularly by the Health Authority to confirm compliance with legal requirements. A similar situation occurs in the case of direct distribution, whereby a Competent Authority authorises a procurement organisation (e.g. HSC collection centre) to send a donation directly to a transplant centre for clinical use without additional processing or storage. In non-EU countries, tissue establishments (tissue banks/cell banks) as well as procurement organisations engaged in direct distribution should be asked to provide evidence of the quality and safety standards they follow, and of any independent quality accreditation or certification they may possess.

Choosing appropriately authorised or certified tissue establishments or procurement organisations helps to ensure that the donors of tissues or cells have been tested and selected correctly, and that all quality system requirements are in place for distribution of the material as well as for possible processing and storage. If the ORHA has a large volume requirement for a particular type of tissue or cell, it may consider it appropriate to conduct a quality audit of the supplier.

Tissues or cells shall not be dispensed without an order from a physician or other authorised health professional. It is good practice for tissue establishments to sign basic end-use agreements with the ORHA before beginning to supply it with tissues or cells. The scope of the agreement should include:

a. details for the contact person and possible forms of communication (phone, fax, e-mail);

b. methods of ordering and delivery of tissues or cells, including liability for transport;

c. conditions of storage and preparation of tissues or cells for use at the ORHA;

d. procedures for disposal of tissues or cells at the ORHA;

e. procedures for return of tissues or cells to the tissue establishment (if permitted);

f. responsibility and procedures for maintaining traceability;

g. procedures for reporting and investigation of adverse reactions and adverse events;

h. procedures for the management of tissue recalls and look-back procedures.

In the case of HSC transplantation, a service-level agreement should be signed between the transplant centre and:

a. the HSC Donor Registry in the case of non-related allogeneic transplantation. This is because, theoretically, the number of registries a single transplant centre is collaborating with could be high. Hence, it is recommended to make an agreement at least with the register the transplant centre is collaborating with most frequently;

b. HSC collecting centre for related allogeneic transplantation;

c. tissue establishment when HSC to be transplanted are processed and/or stored before clinical use (e.g. autologous HSC, cord blood).

Written agreements should be signed, dated, reviewed, and renewed regularly. They must comply with relevant laws and regulations.

For situations in which an ORHA and supplying tissue establishment are within the same healthcare institution, responsibilities should be specified in the Quality System documentation.

It is not recommended for ORHA to obtain tissues or cells with the participation of a broker. If such situations occur, ORHA shall verify that the distributing tissue establishment is authorised or certified appropriately.

Tissue establishments should distribute gametes only to other authorised tissue establishments or OHRA.

10.4. Receiving tissues or cells from other countries

If an ORHA wishes to order tissues or cells from another country, it is good practice to ask a reliable local tissue establishment to locate and communicate with the tissue establishment abroad. Within the EU it is a legal requirement that any imports from third countries are managed by authorised tissue establishments. The only exceptions to this rule are cases of 'direct import distribution'. That is, imported tissues and cells may be distributed directly for immediate transplantation to the recipient as long as the supplier is provided with an accreditation, designation, authorisation or licence for this activity, or in emergency cases. In both of these cases, the Health Authority must authorise the import directly. An example of direct import distribution would be an import distribution of HSC donated for immediate transplantation without storage or processing.

The tissue establishments can liaise to ensure that equivalent standards of safety and quality are applied. For EU member states, any tissue establishment that is authorised in its own member state may provide tissues or cells directly to ORHA in other member states. However, some member states have implemented more stringent rules (as allowed by these Directives) and require formal import procedures to be followed, even if the material comes from another EU country. It is important to be aware of the national legislation in place for the importation of tissues or cells from another country.

10.5. Exceptional release

In exceptional circumstances, ORHA may agree with a tissue establishment or procurement organisation in the case of direct distribution that tissues or cells that do not meet the normal release criteria can be released and used in a specific patient on the basis of a risk–benefit analysis, taking into consideration the alternative options for the patient and the consequences of not providing the tissues or cells. The risk assessment should be documented before acceptance of

the exceptionally released material. The recipient patient's physician should work with the Medical Director of the tissue establishment/collecting centre in conducting the risk assessment and risk–benefit analysis for their patient. These discussions and conclusions should be documented. The treating physician should sign his/her agreement with the exceptional departure from normal procedures where there is any risk implied for the recipient patient. The patient should also participate in (or at least be informed of) the decision process and conclusions before providing consent (see section 10.6).

10.6. Recipient consent

Although donors of tissues and cells are carefully selected and tested, an element of risk remains due to the exceptional nature of the material. Risks can be due to transmission of infection by viruses, bacteria, parasite, fungi or prions, malignancy transmission, hypersensitivity reactions (including allergy, anaphylactic reactions or anaphylaxis), unexpectedly delayed or absent engraftment, toxic effects, unexpected immunological reactions due to mismatch, and transmission of a genetic disease.

In the context of these risks, however small, it is highly recommended that patients who will receive a human tissue or cell be made aware of the facts and give their consent to the risks involved. The Notify Library database led by the World Health Organization and hosted by the Italian National Transplant Centre (www.notifylibrary.org) is a useful tool for accessing risk information for a particular type of tissue or cell. 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 transplant;

b. an estimate of the frequency of the adverse outcomes described;

c. information regarding alternative therapies.

Once the appropriate information has been given, the patient should then provide consent in a form according to national requirements that should include at least the following elements:

a. confirmation that the risks associated with the transplant have been explained and the information has been understood;

b. acceptance of the risks in light of the potential benefits.

This consent form should be separate from the more generic consent to receive treatment or surgery.

10.7. Centralised *versus* decentralised management of tissues and cells

The most prevalent model for the management of tissues and cells in the hospital is that the material is delivered directly to the relevant department or operating theatre (i.e. a decentralised model). The great advantage of the decentralised model is that a specialist clinician is in control. However, maintaining traceability of tissues and cells is very difficult in such a model and compliance with the requirements for storage and handling is also problematic. Centralised models greatly improve the ability to trace tissues and cells and can significantly improve inventory control and compliance with safety and quality standards. For these reasons, a centralised model for the receipt, short-term storage and traceability of tissues and cells for human applications is strongly recommended.

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 hospital or clinic. The roles and tasks of officially designated persons should be clearly specified in standard operating procedures (SOP).

10.8. Incoming inspection at the organisation responsible for human application

The incoming inspection is an important step to be taken if tissues or cells are first received and before they are placed into storage or delivered to the operating room.

ORHA personnel should verify and appropriately record that:

a. Tissues or cells received correspond to what was ordered and to the information in the accompanying documentation, which must be complete and legible;

b. Shipping containers and primary containers are labelled with the information required (see Chapter 12) and labels are affixed and legible. 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. Expiry dates of tissues or cells have been not exceeded;

e. The transport temperature range was monitored or maintained adequately and is acceptable. For tissues or cells that are transported frozen or 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. for refrigerated tissues or cells – wet ice and, for frozen tissues or cells, dry ice). The supplying tissue establishment should be able to provide, on request, a validation study to show that the method of transport is adequate to maintain the required temperature for a certain period of time.

The ORHA should establish a procedure for situations in which the requirements described above are not met.

Moreover, documentation of risk assessment for donors, tissue-related information, and tissue-processing details shall be made available to the transplanting physician upon request, unless such information infringes upon the confidentiality of the donor.

10.9. Package insert/instructions and temporary storage before use

Once tissues or cells have been distributed by a tissue establishment for clinical use, appropriate storage and handling becomes 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 should be followed precisely.

Where a specific storage temperature is necessary from receipt to clinical application, the storage device (fridge, 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 backup 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 a defined period of time (the American Association of Tissue Banks recommends a minimum of 10 years).

It should be ensured that during short-term storage, and before clinical application, the material is stored together with its associated documentation or the documentation should be clearly linked to the tissues or cells and easily accessible. The accompanying document should specify the presence of particular additives or reagents that may affect the recipient (e.g. antibiotics, allergens). This information should be taken into account. If there is no package insert accompanying the tissues or cells, they should not be used.

Some member states within EU recognise short-term storage of tissues or cells as a tissue-establishment activity and do require obtaining specific authorisation from Health Authorities for such activity. There-

fore, it is important to be aware of the national legislation in place for the storage of tissues or cells at ORHA.

10.10. Inspection of the container, documentation and tissues or cells

Before opening the container, or attaching an infusion device to a bag of cells, the accompanying documentation should be 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 what is shown on the label. The packaging and the tissue should be inspected for any signs of damage during transport. Where temperature during transport is critical, there should be confirmation that the required temperature was maintained during transport.

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.

10.11. Preparation of tissues or cells before use

Instructions for opening the container, package, and any required manipulation/reconstitution (e.g. thawing, washing, rehydration), as well as information concerning expiry dates after opening/manipulation and presence of potential harmful residues or reagents that may affect the recipient (e.g. antibiotics, ethylene oxide), must be provided on the label or accompanying documentation of delivered tissues or cells.

The handling instruction should be followed precisely. Any departure from the instructions provided is at the discretion of the clinical user, who will take full responsibility for any adverse outcome resulting from not adhering to supplier instructions.

10.12. Surplus or unused tissues or cells

It is not permitted to use tissues or cells remaining from a clinical procedure in another patient; it should be discarded as clinical or anatomical waste, in accordance with national rules, or returned to the supplying tissue establishment for appropriate disposal. Similarly, a single unit of tissues or cells should not be used in two or more separate patients. Any additional processing of supplied tissues or cells (e.g. its division for use in two or more patients) should be considered as an exceptional departure from normal requirements, and should not be undertaken without notification of the Responsible Person (RP) at the supplying tissue establishment and within the EU without Health Authority authorisation.

Tissues or cells provided to one ORHA should not, in general, be sent to another entity for clinical use. Within the EU, this would be defined as 'distribution' and it would require specific authorisation. Tissues or cells that are received and not subsequently used in one department of a hospital may be sent to a different department or operating theatre in the same hospital but, as a minimum, the supplying tissue establishment should be informed of this. There may be nationally established rules for this scenario.

The documentation that accompanies the tissues and cells should specify whether they can be returned to the tissue establishment if not opened or used, e.g. if the patient is not well enough for surgery or surgery is cancelled for another reason. Most tissue establishments will not accept tissues or cells that are returned under these circumstances. Tissue establishments that do accept returned, unused and unopened tissues or cells will have to confirm that the conditions for maintenance of the required properties of tissues or cells were provided and documented continuously, and that the packaging was not tampered with.

10.13. Traceability

Coding and traceability are addressed fully in Chapter 13. In the EU, ORHA are required to maintain traceability records from the point

of receipt of the tissue until 30 years after clinical use or other final disposal. These records (mandatory in the EU) must include:

a. identification of the supplying tissue establishment or collecting centre;

b. identification of the clinician/end user/facility;

c. type of tissues or cells;

d. product identification;

e. identification of the recipient;

f. date of application.

Details of the tissues or cells applied should be in the recipient's hospital record and in the logbook of the operating theatre where they have been applied during transplantation. Alone, this is not adequate to permit rapid re-tracing of patients who might be at risk from a particular donation or processing batch. ORHA should also have an electronic or paper 'log' where all received, transplanted and discarded tissues or cells are recorded. This will allow quick and easy action in the case of a 'recall' by the tissue establishment or the Health Authority or for internal follow-up/review. 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 safe storage should be clearly identified and documented.

Some supplier tissue establishments require 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 medical record. The details should be sufficient to unambiguously identify the recipient, i.e. at least three points of identification including a unique identifier. 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 other final disposal. Where cards or forms are returned to the supplier tissue establishment, the manner of documentation should adhere to national data protection regulations and should ensure that personal information

is not visible or that the recipient's privacy is not compromised in any way.

It is highly recommended that when patients 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/unusual findings to the ORHA.

10.14. Adverse events and adverse reactions

Vigilance and Surveillance (V&S) are addressed in full in Chapter 14. Effective V&S relies heavily on all health professionals involved from procurement to transplantation, but particularly medical staff (including surgeons) involved in tissue and cell procurement activities that might be aware or informed of additional safety information on donors during their follow-up.

Serious adverse reactions (SAR) can be detected during and after donation in living donors or after transplantation in tissue or cell recipients. As adverse transplant outcomes might result from many diverse factors associated with the clinical procedure or the patient's underlying condition, clinicians might not consider the tissues or cells transplanted as a possible reason for the outcome. Tissue establishments 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 any of the stages from donation to clinical application so that similar occurrences might be prevented in the future.

For most types of well-established tissue and cell transplantation, detailed clinical outcome reporting by the clinical user to the tissue establishment is required only in those exceptional circumstances where there is an untoward serious adverse reaction. Routine clinical follow-up and reporting of tissue and cell recipient clinical progress is

required for all highly-matched life-saving transplants, such as HSC 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 generally considered as part of vigilance.

Tissue establishments that supply tissues and cells should provide clinical user organisations with clear instructions on how to report SAR, preferably using standardised documentation. In general, suspected SAR should be reported by the clinical users to the tissue establishment that supplied the tissues or cells immediately, before it is confirmed or investigated, to enable the tissue establishment to take appropriate precautionary actions to prevent harm to other patients and to involve the tissue establishment in the investigation process. As described further in Chapter 12, the clinician treating the recipient plays a critical part in the investigation of suspected SAR, together with the supplying tissue establishment.

10.15. Management of recalls and reviews

There are various reasons why a tissue establishment may recall tissues or cells that were distributed to an ORHA. A recall may be related to the receipt of new information regarding the donor's medical or behavioural history that implies a risk of disease transmission risk or 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 tissue establishment or required by the national Health Authority.

When a tissue establishment 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 log-book or electronic database 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 scenario 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 will facilitate effective action.

A review may 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. In this case, a central log-book or database of the tissues or cells supplied will also greatly facilitate the process.

10.16. References

1. Guidelines for Healthcare Professionals on Vigilance and Surveillance of Human Tissues and Cells Part 1 – Tissues, Part 2 – Haematopoietic Stem Cells, Deliverable 10 of Project SOHO V&S, available at www.notifylibrary.org/sites/default/files/SOHOV%26S%20Vigilance%20Guidance%20for%20Healthcare%20Professionals%20-%20Part%201%20Tissues_0.pdf, accessed 27 May 2015.
2. Exploring Vigilance Notification for Organs, Tissues and Cells, Notify, available at www.notifylibrary.org/sites/default/files/SOHO%20V%26S%20Vigilance%20Guidance%20for%20Healthcare%20Professionals%20-%20Part%202%20HPCs_0.pdf, accessed 27 May 2015.

Chapter 11

Computerised systems

11.1. Introduction

Computerised systems are playing an ever-increasing part in the management of business operations, including those related to healthcare. Tissue establishments and donor registries may use a wide range of computerised systems. These can range from simple stand-alone computer systems that use a software package to track and trend data, to fully integrated systems that control a range of processing steps and present data that allows release of tissues and cells for clinical applications. In some cases they are relied upon to record consent and donor identity. Computerised systems may also have a role in controlling the facility or ensuring that required environmental conditions (e.g. air pressure differentials, particle counts) are maintained (e.g. building management system).

Errors and malfunctions of computer systems can go unnoticed and might have serious consequences. Changes in software must be managed carefully to ensure that data have not been corrupted or reorganised in a manner that changes their meaning or impact. A review conducted for the UK Secretary of State for Health published in 2010 reported the discovery of a systematic error in the documentation of wishes of organ donation that had probably occurred in 1999

and which potentially affected the records of >900,000 individuals [1]. Computerised systems help to bring efficiency to processes. However, if they record critical information with an impact on donation, processing and release of tissues and cells, they must be selected and validated just as any other piece of critical equipment [2].

11.2. Planning the implementation of a computerised system

Before implementation of a computerised system, the user should:

a. undertake a domain analysis to understand the context of use of the system;

b. identify and put in place the required infrastructure;

c. establish a list of requirements that will meet their needs, including the duration of record storage (in general, 10 years is required for quality system-related data and 30 years for traceability-related data in the European Union [EU]);

d. evaluate the different systems available and choose the one that meets the established requirements;

e. audit the developer/manufacturer to ensure that they can provide a product that meets regulatory requirements;

f. establish responsibility between the user and developer/supplier/manufacturer to define roles and responsibilities with regard to testing, user instructions, maintenance, system improvements and access to source codes.

These steps ensure that the user has all the necessary information about the purchased system and has an established relationship with the developer. 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 (e.g. manuals, standard operating procedures [SOP]). To define the system, in co-operation with the vendor or developer, the

user should generate a written description of the system, the functions that it is designed to carry out, and all human interactions (functional and non-functional requirements). These requirements will be the basis for subsequent testing and validation of the developed/supplied system. For further information, refer to ISO/IEC 12207 and ISO/IEC/IEEE 29148 [3, 4].

11.3. Qualification and testing

Guidance on the qualification of new equipment in Chapter 2 should be taken into account. The qualification of computerised systems in a tissue establishment should be incorporated in the general validation plan of the centre that should include:

a. the identity of the computerised systems and interfaces that are subject to validation;

b. a brief description of the qualification strategies for different categories of computerised systems, as well as other validation activities;

c. an outline of the protocols and related test procedures for all validation activities of the computer system. The reporting requirements for documenting the validation exercises and related results should also be defined;

d. the identity of key personnel and their responsibilities as part of the validation programme.

The level of qualification required for computerised systems is dependent upon the criticality of the systems to the quality and safety of the tissues and cells. Thus, a criticality rating should be applied to all computerised systems in place. The method of validation of these critical systems is dependent on the type/category of software used. Table 11.1 gives some examples with suggested approaches of validation.

Table 11.1. Approach to qualification and control of computerised systems by system category (modified from the International Society for Pharmaceutical Engineering [ISPE] [5])

Category	Description	Typical examples	Typical approach
1. Infrastructure software	• Software upon 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
2. Non-configured	• Software cannot be configured to suit the specific process	• Firmware-based application • Commercial off-the-shelf software packages • Instrument software (e.g. software associated with machines used for testing bacteriology or serology, cell counters)	• Specification of 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

Category	Description	Typical examples	Typical approach
3. 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 supplier has adequate quality management system • Some lifecycle documentation retained only by supplier (e.g. design specifications) • Record version number and verify correct installation • Risk-based testing to demonstrate application works as designed in a test environment • Risk-based testing to demonstrate that the application works as designed within the routine environment • Procedures in place for maintaining compliance and fitness for intended use • Procedures in place for managing data
4. 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 building management systems (monitoring air pressures in rooms, temperature and/or particles, temperatures of fridges, freezers and incubators) • Clinical trial monitoring	Same as for 'Configured' above, but also: • More rigorous supplier assessment, with possible supplier audits

Qualification should be commensurate with level of risk-intended use and potential implications of malfunction to quality and safety.

Before qualification of a newly installed computerised system can be carried out, a full set of documentation that is as detailed as necessary to ensure appropriate operation of the system must be in place. The documentation should include:

a. a detailed specification (inventory) of the hardware, software and peripheral devices, including their environmental requirements and limitations;

b. diagrams or flowcharts of the system's operations that describe all component interfaces, a network diagram (if applicable) and all database structures (e.g. file sizes, input and output formats);

c. SOP that describe how the system is used. The user should develop the SOP based on the instructions for use provided by the software developer and the internal procedures of the establishment. In particular, SOP should address all manual and automated interactions with the system, including:

   i. routine back-up, maintenance and diagnostic procedures, including assignment of responsibilities;

   ii. safety leading indicators [6];

   iii. 'work-arounds' for system limitations;

   iv. procedures for handling errors, including assignment of responsibilities;

   v. procedures for handling disasters and contingency planning, including assignment of responsibilities;

   vi. procedures for supervised changes to incorrect data;

   vii. procedures for validation of a change;

   viii. a training system that includes training manuals, documentation and procedures for training.

Qualification documents and the results of tests undertaken and approved by the supplier/vendor/developer of the system should be

presented to the user. The user can then carry out tests according to a pre-defined and documented test plan [7]. Types of risk to consider include inadequate design of a system, errors that may occur in use (errors of use or system defects), and loss or distortion of data [8]. Testing should involve the entire system, and in the manner in which it is expected to perform routinely in the establishment. Testing may be done by a third party but, in that case, must also include personnel from the tissue establishment. The following types of basic testing should be conducted:

a. Functional testing of components

   i. System components are presented with all types of expected interaction, including normal value, boundary, invalid and special case inputs. The system must produce the correct outputs, including error messages by control programs. Carrying out such testing in parallel with a reference or standard system can be useful;

   ii. Each test case should include the input, expected output, acceptance criteria and whether the test passed or failed. For traceability purposes and to facilitate quality assurance review and follow-up, it is recommended that any supporting documentation (e.g. print screens) be included to verify the specific test case.

b. Data migration

The process for data migration should be defined, documented and tested appropriately. This should ensure full maintenance of traceability, including archiving of data (if necessary).

c. Environmental testing

In the actual operating environment, functional tests are carried out to demonstrate that:

   i. the software systems work appropriately with the hardware;

   ii. all applications of the software perform appropriately with the software operating system;

iii. appropriate information passes correctly through system interfaces, including appropriate data transfer to or from other laboratory and automated (e.g. serology testing, cell counting) systems (if applicable);

iv. accessories such as barcode scanners perform as expected with the barcodes in use (if applicable);

v. printed reports are formatted appropriately and correctly;

vi. personnel are trained and use the system correctly;

vii. the system performs appropriately at peak production times and with the maximum number of concurrent users;

viii. back-ups restore data in a correct way.

11.4. Change control

In case of changes in the software, the validation status needs to be re-established. If a re-validation analysis is needed, it should be based on risk assessment and conducted not only for validation of the individual change, but also to determine the extent and impact of that change on the entire computerised system.

11.5. Maintenance of the system

The database should be checked periodically and systematically by qualified Information Technology (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.

Security should be maintained by:

a. an adequate change history of the system, including for 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.

11.6. Quality assurance

The data-processing system should be considered as critical equipment within the quality-assurance programme. As a minimum, such oversight should include:

a. ensuring the ongoing accuracy and completeness of all documentation on equipment, software maintenance and operator training;

b. undertaking audits periodically to verify appropriate accomplishment of all performance tests, routine maintenance, change procedures, data-integrity checks, error investigations and operator-competency evaluations.

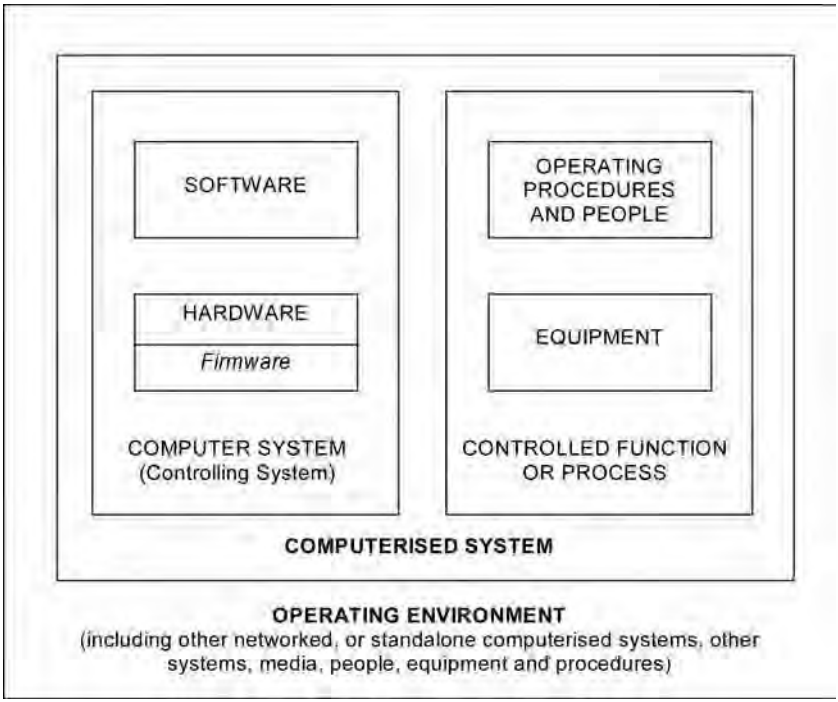
11.7. Industry guidance for validation of the computerised system

The most common industry guide used for validation of computerised systems is that from the International Society for Pharmaceutical Engineering (ISPE) [2]. More specific guidance related to blood and tissues is available from the British Committee for Standards in Haematology [9].

11.8. Regulations governing validation of computerised systems in Good Manufacturing Practices

Regulation of computerised systems is well established in the pharmaceutical industry, with European Good Manufacturing Practices (GMP) [10] acting as the regulatory reference in Europe. *Pharmaceutical Inspectorate Co-operation Scheme Guidance (PIC/S)* [11] is also used by inspectors in the EU. The pharmaceutical industry operates on a global scale, so many European companies maintain compliance with the US Food and Drug Administration (FDA) [12]. These regulatory documents can be useful sources of reference for tissue establishments. In its guidance, *PIC/S* has represented how computerised systems must interact with their environment [5] (Figure 11.1).

Figure 11.1. Relationships between the various components of a computerised system in its operating environment (from *'PIC/S Good practices for computerised systems in regulated GxP environments* [*PI 011-3*, currently under revision])' [11])



If a computerised system replaces a manual operation, there should be no resultant decrease in product quality, process control or quality assurance. There should be no increase in the overall risk of the process [10].

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 [10].

11.9. Infrastructure

Infrastructure should include consideration of design and development tools (e.g. test suites, servers, version- and configuration-control systems, modelling and architecture tools, communication tools, traceability, and behavioural-modelling tools)

11.10. Failure of the system

For computerised systems that support critical processes, provisions 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 [10]. Testing of these alternative systems and their ability to retrieve data should be assessed annually.

11.11. Electronic signature

Records may be signed electronically. According to *Annex 11 of EU GMP* [10], 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.

11.12. Data protection

All personal data stored in computerised systems must be stored in a secure manner with access available only by authorised personnel. Procedures for personal data protection must comply with national legal requirements and, for EU countries, with the requirements defined in Directive 95/46/EC *on the Protection of individuals with regard to the processing of personal data and on the free movement of such data*.

11.13. Archiving

Critical data must be archived in a long-term stable medium and placed 'off-site' at a location remote from the hardware to ensure secure storage. This data should be checked for accessibility, readability and integrity. If changes are made to the system (e.g. new computer equipment or software are installed), then the ability to retrieve archived data must be ensured and tested [10].

11.14. References

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12. Title 21 Code of Federal Regulation (CFR) Part 11, United States Food and Drug Administration, available at www.fda.gov/regulatoryinformation/guidances/ucm125067.htm, accessed 27 May 2015.

Chapter 12

Packaging and labelling

12.1. Introduction

Packaging of tissues and cells has an important role during all banking procedures, starting from procurement through processing steps, distribution and clinical use. Adequate packaging minimises the risk of contamination of tissues and cells as well as the persons involved in transportation, and ensures its required characteristics and biological function.

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 these stakeholders participate and contribute to actively safeguarding, in a continuous manner, the tracking of the stages tissues and cells go through. Accurate tracking of tissues and cells allows reliable data to be scientifically assessed for potential risks to the donor, to the procurement and processing operations and to the storage, transport and clinical use of donated material. Traceability is addressed in depth in Chapter 13. An essential aspect of ensuring accurate traceability is clear and complete labelling of tissues and cells at all stages. The system of identification for donors and recipients must be aligned with the packaging and labelling system of tissues and cells in such a way

that a correlation between tissues and cells, the source and the recipients exists at all times.

Labels must be attached to packaging that has been validated to demonstrate that it maintains the required properties of the tissues and cells and ensures integrity. This chapter addresses good practices in packaging and labelling at all stages from donation to implantation.

12.2. General concepts

Packaging and labelling operations must be considered an integral part of tissue-establishment activities and procurement organisations. It must be included in the training of personnel and specified in all relevant procedures. Although this chapter establishes specific recommendations for packaging and labelling for the procurement and processing phases, they should equally apply to intermediate phases, such as in-process steps, where all materials, containers, equipment and unfinished tissues and cells must be adequately identified at all times. In addition, tissues and cells obtained and/or processed for research purposes should be clearly identified as such on their packages and labels (e.g. 'FOR RESEARCH USE ONLY' or 'NOT FOR CLINICAL USE').

There should be written procedures describing the receipt, identification, quarantine, sampling, examination and/or testing and release, as well as handling of packaging and labelling materials.

Premises for the packaging and labelling of tissues and cells as well as the operations carried out must be designed to prevent any cross-contamination or mix-ups. For the same reason, simultaneous operations must be avoided (or at least adequate measures should be taken).

Primary packaging and labelling of tissues or cells must be done in a qualified environment, which must be specified in standard operating procedures (SOP).

For European Union (EU) member states, the requirements for packaging and labelling of tissues and cells are detailed in Annex IV of Directive 2006/17/EC and Annex II of Directive 2006/86/EC.

12.3. Packaging of tissues and cells

Packaging includes all operations, including primary and secondary packaging, which procured or processed tissues and cells undergo to become starting, in-process or finished packaging. Packaging aims to protect tissues and cells, and to present it to the operator (for starting or in-process packaging) or to the clinical user (for final packaging) in a suitable manner. The type of substances of human origin and its intended use will determine the requirements needed to carry out a packaging operation in a safe manner. Special consideration must be made for the primary packaging that will be in direct contact with tissues and cells. Containers intended to be used as primary packaging should be submitted to visual inspection before use and the need for applying an adequate cleaning process should be assessed along with suitable sterilisation methods such as irradiation or autoclaving of materials and containers. These packaging materials should be stored in a clean area. In this case, the materials and the conditions under which packaging takes place must be carefully specified, assessed and approved before use. Processing facilities must establish and document validated packaging protocols.

Packaging must ensure the integrity and maintain the sterility of the contents of the primary container. Storage containers must be appropriate for the type of tissue or cells, the temperature and type of storage method and the intended application. They must withstand sterilisation (where this is to be applied), not produce toxic residues during storage and be adequately robust to remain intact when handled during transport. Each tissue or cell container must be examined visually for damage or evidence of contamination before distribution for clinical use.

12.4. Labelling of tissues and cells

Written procedures must be established and followed to ensure correct labelling. Each labelling phase for all tissues or cells must be documented. Material must be labelled during all phases of procurement,

processing, storage and distribution. Labelling must be clear, legible, indelible and unique.

Before labelling a unit of donated or processed material, the container must be inspected for evidence of impurities, defects, broken seals or contamination that could compromise the quality, integrity or safety of the tissue or cells.

Labels attached to the containers should identify and describe the contents. The description should characterise the tissues and cells and reflect key aspects with regards to their maintenance and use. Standard nomenclature and standard international units of measurement must be used to describe tissues and the processing they have undergone.

Identification should provide information on traceability that links the tissues and cells to the tissue establishment of origin and, ultimately, the donor. When tissues or cells are to be distributed internationally, language barriers should be considered and information translated or coded to enable understanding.

For EU member states, the primary label of distributed tissues and cells shall include the Single European Code (SEC) for tissues and cells as detailed in Commission Directive (EU) 2015/565.

For autologous or directed donations, the name and/or identifier of the intended recipient must be included in the label.

Further guidance on traceability and coding is provided in Chapter 13.

The production of labels must be controlled. When applicable, reconciliation of labels that have been edited, used and/or returned/rejected must be undertaken according to written procedures. All excess labels containing quality or traceability information must be destroyed or maintained in a secure manner, when necessary, to prevent mix-ups. Obsolete unused labels must be destroyed according to written procedures.

It is highly recommended to undertake labelling and packaging simultaneously, in a continuous process, to reduce the risk of mix-ups or cross-contamination. Before application to the container, printed

labels must be carefully examined to ensure that the information they contain conforms to the corresponding tissues or cells. The results of this examination should be documented at identified critical stages. Labels must be designed to adhere firmly to the container under all anticipated storage and transport conditions. The label applied by the facility must not be removed, altered or obscured. A sufficient area of the container must remain uncovered to permit inspection of the contents, whenever possible.

For processing of batches that include large numbers of individual final units, a representative printed label should be included in the processing batch record.

12.5. Sample and documentation labelling

All key blood and tissue samples for testing or archiving and all related documents must be labelled in a legible, indelible and unique manner that ensures traceability to the donor and the associated donations. A record of the time and place the sample was taken must be included on the label or in accompanying documentation.

12.6. 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 'qualified', and they must be suitable for use with biological 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 main-

tain integrity and remain attached to primary packages and transport containers at the storage temperatures.

All printed labels for primary and secondary packaging and leaflets should be segregated and stored in access-controlled areas.

Management of packaging and labelling materials must include the following:

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 and/or testing, release, and handling of 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 later version is attached to the tissue or cells should be described in a written manner;

d. a study must be done to document the suitability of packaging material, containers and labels for their intended purpose.

12.7. Primary packaging and labelling for procurement operations

'Primary packaging' refers to the materials that are intended to come into direct contact with the tissues and cells and are, therefore, considered to be 'critical'. Selected materials should not leach harmful chemicals, they should be capable of being sterilised by a safe method if appropriate, and be sealable and leak-proof.

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 function of the tissues and cells.

Packaging must also prevent contamination of those responsible for packaging 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 is packed separately in sterile packaging as soon as possible after recovery. Double or triple wrapping is necessary, depending on the tissue-specific requirements. Musculoskeletal tissues and skin may be packed in sterile, transparent polymer foil, but additional cotton wrapping can be used. Corneas shall be placed in sterile transparent containers with medium, whereas heart, amniotic membrane, whole eyeballs, skin or cartilage for cell cultures shall be packed in sterile containers with transport medium. Composition of the transport medium for a particular type of tissue shall maintain the biological properties of tissues, and shall include antibiotics and antimycotics validated by type and concentration.

A unique identification code shall be allocated to the donation and donated tissues and cells during procurement, at the end of the recovery process or in a tissue bank to ensure appropriate identification of the donor and traceability of all donated material. Coded data shall be entered in a register maintained for this purpose.

The minimum required information that must be present in a primary label shall include:

a. identification of donor or donation;

b. type of tissues or cells packed;

c. date of donation (and time, where possible);

d. applicable hazard warnings;

e. description of any additives (if used);

f. in the case of autologous donation, the label must state 'FOR AUTOLOGOUS USE ONLY';

g. in the case of directed donation, the label must identify the intended recipient;

h. if applicable, the fluid in which the tissues or cells are preserved.

If any of the information listed above cannot be included on the primary package label, it must be provided in accompanying records inside the transport container.

12.8. 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 the required information are not attached to the primary packaging they should be attached to the secondary packaging which should be closed and sealed.

12.9. Outer container packaging and labelling for procurement operations

Packaged tissues and cells must be shipped in a container which is suitable for the transport of biological materials and which maintains the safety and quality of the tissues or cells contained within. Temperature conditions between recovery and processing must be appropriate to the type of tissue so as to preserve its required characteristics and biological function (i.e. temperature and duration of transport to the tissue establishment where the tissue processing will take place). The container must be closed fully and not opened until the procured tissues or cells are received by the tissue bank.

When tissues or cells are shipped from the procurement site to the tissue establishment (rather than taken by the tissue establishment team or procurement team), the transport container must be labelled with:

a. 'HUMAN TISSUE' or 'HUMAN CELLS' and 'HANDLE WITH CARE';

b. the name, address and telephone number of the procurement organisation from which the package is being sent and a contact person in the event of problems;

c. the name, address and telephone number of the tissue establishment (and a person to be contacted to take delivery of the container) or identification information regarding the clinical team responsible for receipt of the container if the tissues or cells are being transported for immediate application, the date and time of the start of transportation, and specifications concerning conditions of transport relevant to the quality and safety of the tissues and cells;

d. specifications concerning conditions of transport relevant to the quality and safety of the tissues and cells (e.g. 'DO NOT DELAY', 'KEEP COOL' and 'KEEP UPRIGHT');

e. in the case of viable cellular products, use the warning 'DO NOT IRRADIATE';

f. when cells or tissue are known to be from a donor that has tested positive for a relevant infectious disease, the warning 'BIOLOGICAL HAZARD' should be used;

g. specifications concerning storage conditions (such as 'DO NOT FREEZE');

h. in the case of an autologous donor, the indication 'FOR AUTOLOGOUS USE ONLY' should be used;

i. any other applicable hazard warnings.

12.10. Procurement package insert

It is recommended that documentation sent with procured tissues or cells to a tissue establishment indicate, where applicable, that they are in a state of 'quarantine' so 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.

12.11. Packaging and labelling during processing

Packaging and labelling of unfinished tissues and cells during intermediate phases of processing shall be applied to all packaging materials and containers to assure identification at all times.

12.12. 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 12.7., with a special focus on the radiation-resistance of packaging material for tissue that will be radiation-sterilised. The expiry date will be determined by the properties of the tissues and cells, but also by the integrity and stability of the packaging and labelling materials, among other factors.

Packaging and labelling procedures shall be done to prevent cross-contamination or mix-ups. Simultaneous operations should be avoided or adequate measures should be taken to ensure no cross-contamination or mix-ups occur.

Facilities where packaging or labelling operations have taken place should be checked before starting any other operation so as to guarantee that all previous materials have been removed.

Printed labels should be examined carefully to ensure that the information contained conforms to the corresponding tissue/cells. Results of this examination should be documented. A printed label, representative of those used, should be included in the processing records.

Unused and already printed labels shall be destroyed according to written procedures.

The primary tissue/cell package label must provide:

a. identification number or code;

b. lot number or batch number, if applicable (see Chapter 13);

c. type of tissues and cells;

d. identification of the tissue establishment;

e. expiry date for particular defined storage conditions;

f. in the case of autologous donation, the indication 'FOR AUTOLOGOUS USE ONLY', together with the identification of the donor/recipient;

g. in the case of directed donations, the label must identify the intended recipient;

h. when tissues and cells are known to be positive for a relevant infectious disease marker, the package must be marked as 'BIOLOGICAL HAZARD';

i. if applicable, product modifiers, manipulations and additives used;

j. in case of distribution in EU member states, the SEC should be printed.

Information regarding the SEC can be found in a Chapter 13.

The following information must be provided either on the label or in accompanying documentation:

a. description (definition) and, if relevant, dimensions of the tissue or cell product;

b. morphology and functional data, where relevant;

c. date of distribution of the tissues and cells;

d. biological determinations carried out on the donor and results;

e. storage conditions required;

f. instructions for opening the container or package and any required manipulation/reconstitution;

g. expiry dates after opening/manipulation;

h. procedures to be applied before use (e.g. rehydration, thawing);

i. instructions for reporting serious adverse reactions and/or events;

j. presence of potential harmful residues (e.g. antibiotics, ethylene oxide);

k. if applicable, the fluid in which the tissue is preserved;

l. any other applicable hazard warnings.

12.13. 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 taken 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.

Information in section 12.11 should be included on a label on the secondary packaging if, for some reason (such as the size or material of the primary packaging), it cannot be included on the primary packaging.

12.14. Outer container packaging and labelling for finished tissues and cells

When tissues or cells are shipped for distribution purposes, every transport container must be guaranteed to maintain the conditions needed for the specific tissue or cell type. Containers should provide adequate protection against deterioration or contamination of tissues and cells that may occur during storage and transportation. Containers should be clean and sanitised before use to ensure that they are suitable for their intended use. These containers should not alter the quality, safety and efficacy of tissues and 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 at least the following information:

a. identification of the source tissue establishment, including an address, telephone number, and contact person (if possible);

b. identification of the destination organisation (organisation responsible for human application – see Chapter 10), including an address, telephone number and contact person (if possible);

c. a statement that the package contains human tissue or cells and the warning 'HANDLE WITH CARE';

d. if living cells are required for the function of tissues or cells (e.g. stem cells), the warning 'DO NOT IRRADIATE' must be added;

e. recommended transport conditions (e.g. 'KEEP COOL' and 'KEEP UPRIGHT');

f. safety instructions for the method of cooling (where applicable);

g. any other applicable hazard warnings;

h. if transport is 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 shipment. The lower-half of the label must indicate the external transportation range of the shipment in degrees centigrade (for more details, see section 12.16).

12.15. Package insert for finished tissues and cells

A 'package insert' refers to the complementary information associated with tissues and cells that cannot be placed on labels. Critical information for the clinical user must be provided, including the following:

a. date of distribution;

b. a statement that the tissues or cells are suitable for transplantation following all required disease screening and testing;

c. instructions for proper storage, and thawing where appropriate, must accompany all tissues and cells. They should address preparation, handling and reconstitution where appropriate;

d. inserts should also contain the following information:

   i. a statement limiting use of the material to specific health professionals;

ii. a statement that the material is intended for single use in one patient only;

iii. any applicable hazard warnings;

iv. instructions for opening the package and/or container, and for reconstitution or any other preparation of the contents (if appropriate);

v. the expiry time (if applicable) of the tissue or cells following reconstitution;

vi. a statement, if applicable, that the tissues or cells may not be sterilised or re-sterilised;

vii. recommended storage conditions;

viii. a statement that it is the responsibility of the end user to maintain the tissue or cells in appropriate storage conditions prior to transplantation;

ix. special instructions as appropriate, e.g. 'DO NOT FREEZE';

x. a statement if known sensitising substances are present (including type of antibiotics and preservatives added during processing that might be present as residues);

xi. a statement that adverse reactions potentially attributable to the tissue or cells will be reported promptly to the tissue establishment;

xii. a statement that it is the responsibility of the recipient hospital or other material storage and distribution facility, or the clinician, to maintain recipient records for tissue tracking and post-transplantation follow-up.

For monitoring of transport period and conditions, a data logger may be inserted into the transport container.

12.16. Customs clearance

For clearance of customs, all tissues and cells supplied from abroad require a clear description of the content of the consignment, its des-

tination and intended use. The paperwork sent with the consignment should include the World Customs Organisation Tariff Number for Human Tissue for Transplantation, which at present is 30029010, but there is a request that this should be altered to base code 082 with subcodes. It is important that frozen tissues and cells which are usually packed in dry ice or stored in liquid nitrogen or in a dry shipper must not be delayed at border crossings. If the goods are being transported by air, packages must be labelled with the appropriate IATA codes (UN 1845 for dry ice or UN 1977 for liquid nitrogen in a dry shipper). Therefore, it may be expedient for the importer to inform Customs of a prospective consignment and any enquiries by Customs should be answered promptly. The agreement with the exporting tissue bank should define responsibilities for meeting the cost of transport, refrigeration and/or storage at a Customs facility for any items that may be detained pending customs enquiries.

12.17. References

1. Good tissue practices, Euro-GTP Guidance, available at www.eurogtps.com, accessed 27 May 2015.
2. Handling labels, International Air Transport Association, available at www.iata.org/publications/dgr/Pages/handling-labels.aspx, accessed 27 May 2015.
3. IATA perishable cargo regulations, chapter 17 (amended by Resolution 607 in 2012), International Air Transport Association.

Chapter 13

Traceability

13.1. Introduction

Clinical use of tissues and cells brings great benefits for patients. There are, however, rare (but important) risks associated with such clinical use, including graft failure and donor-transmitted infections and malignancies. The concept of traceability has two main components: (i) it is the means to link a donor with a recipient, or multiple recipients, and (ii) it is the way to identify and link all the steps and procedures to which tissues or cells are subjected, together with their location and the equipment and materials used, before they reach the recipient. Traceability is essential to ensure 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 is the means by which legitimate donation with proper consent can be verified for every tissue or cell product.

Donor selection, procurement from the donor, and thorough processing, storage and distribution (transport) of tissues and cells involve many complex steps that determine the quality of the tissues and cells used for clinical application. Human error, equipment failure, use of inadequate written procedures or new risks that cannot

be predicted, may affect the safety or quality of tissues and cells at any of these stages, which could in turn increase the risk to recipients. 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 assisted reproductive technology (ART), traceability does not stop when the tissues and cells reach the recipient. The health of the children born as a result of ART treatment must be followed up, so identification of children is included in the chain of traceability.

Tissue establishments must be able to locate and recall tissues and cells once they become aware of information that may have implications for their quality and safety. 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' [1]. 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, and the existence of international markets for equipment, consumables and additives adds to the need for robust systems of traceability.

Traceability underpins biovigilance (see Chapter 12). Within each tissue establishment, investigation of adverse events and adverse reactions, and of deviations from standard methods, can be carried out only if a system of traceability is in place. Many establishments share practices, and effective investigations can help to improve shared practices and improve standards. Hence, in addition to biovigilance,

ongoing quality improvement of procedures and practices relating to procurement, processing, donor testing, storage and distribution of tissues and cells also relies on good systems of traceability.

13.2. What is traceability?

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. 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 from the donor) and all records associated with the donation, processing, storage and distribution of the final products and all samples taken from the donor or from the donation for the purposes of testing for quality and safety;

c. the recipient of the tissues or cells;

d. the health of the resulting children (for ART treatment).

The following are key requirements of an effective traceability system:

a. Unique identification

At each stage in the pathway, from donor to recipient or child conceived as a result of ART treatment, each organisation holding tissues or cells must have records on the donor, the donation and donation samples, and must ensure that they are identified and labelled uniquely within their own organisation. While uniqueness can be ensured without difficulty within one organisation, the risks of duplication are increased when tissues, cells, samples or records move from one organisation to another. For example, duplicate identifiers may result when samples are sent to a testing laboratory or when tissues or cells are sent to a hospital because each receiving establishment may assign its own identifier. This risk can be eliminated if a global standard is used to identify samples or tissue products. Avoiding duplication of identifiers can involve including a tissue establishment

or procurement organisation identifier as an element of the identification for the donor, donation or sample. Within the European Union (EU), the European coding system will help to address this need (see section 13.4).

b. Safe transfer of critical information

The 'traceability trail' depends on the accurate transcription of critical identification information. Manual transcription errors cause breaks in the traceability trail. Use of electronic transfer of critical information (bar codes or other machine-readable codes) is recommended. If manual transcription is used, a double-blind entry system should be implemented.

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 ART. A delay could result in harm to patients or children conceived through ART. 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

It is essential that each organisation in the chain clearly understand its responsibilities for traceability. It is notable that in the high profile cases of viral transmission during transplantation that have been published, hospitals were often not able to trace all recipients [2].

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 transplantation 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 ≥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 pro-active approach to prevent obsolescence. The location of traceability records may change when organisations merge 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.

f. 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.

13.3. Which records must be traceable?

There must be a system of record-keeping relating to activities associated with tissues and cells. Records should describe procurement, donor testing, processing, storage, distribution and end use. Records should include 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.

Tissue establishments must ensure that data protection and confidentiality measures are in place in accordance with the data protection laws in the relevant country. Many organ donors are also tissue donors 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 10) and there must be procedures to back up electronic records to prevent loss, corruption and unauthorised access and 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, cryopreservatives and packaging materials used during procurement and processing. The tissue establishment should also retain temperature records, analyser print-outs 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 relevant information retained abroad remains accessible for as long as it may be required.

The records must be kept in an appropriate archive, which is acceptable to the Health Authority of each respective country.

13.3.1. Records of identification, donor tests and clinical evaluation of the donor

Donor records must contain at least the following:

a. donor identity;

b. age, sex, medical and behavioural history of the donor;

c. outcome of physical examination for deceased donors;

d. completed haemodilution formula (where applicable);

e. consent/authorisation form;

f. clinical data, laboratory test results and the results of any other tests carried out;

g. for deceased donors, the results of the autopsy (if carried out) or preliminary verbal report;

h. for haematopoietic stem cell (HSC) donors, the donor's suitability for the chosen recipient;

i. for unrelated HSC donations, where the organisation responsible for procurement has limited access to recipient data, the end user or his/her physician should be provided with the relevant donor data to confirm suitability.

In addition, the donor testing records must contain at least:

a. date and time donor blood samples were taken (blood samples from deceased donors must be taken <24 h of death);

b. date of receipt of the blood sample at the testing facility (in-house or at a contracted laboratory);

c. record of each test kit used to test donor blood sample (i.e. manufacturer, lot number, expiry date);

d. results of donor testing, including repeat testing (if applicable).

13.3.2. Records of procurement of tissues and cells

The organisation undertaking procurement should produce a procurement report and provide it to the tissue establishment. The procurement report should contain at least:

a. the identification of the tissue establishment receiving the tissues or cells;

b. donor identification data (including how and by whom the donor was identified);

c. description and identification of procured tissues and cells (including samples for testing);

d. identification of the person who was responsible for the procurement session, including his/her signature;

e. date, time (start and end, if relevant) and location of the procurement and standard operating procedure used;

f. description of the physical area where procurement took place, including environmental conditions at the procurement site;

g. for deceased donors, storage conditions of the deceased donor, i.e. refrigerated (or not) and time of start and end of refrigeration;

h. manufacturers and lot numbers of reagents and transport solutions used;

i. any incidents that occurred during procurement.

13.3.3. Records of processing of tissues

The organisation undertaking processing should keep the following records (non-exhaustive list):

a. tissues and cells received and evaluation of their suitability;

b. standard operating procedures used to process the tissues and cells;

c. equipment used during processing;

d. records of consumables used during processing (manufacturer, lot number, storage conditions of consumables [if appropriate], expiry date);

e. records of sterilisation or decontamination, if applicable;

f. records of cryopreservation and freezing protocols, if applicable;

g. records of environmental monitoring (temperature monitoring, microbial monitoring and particle counts as appropriate);

h. records of product testing, including microbial testing;

i. any incidents that occurred during processing.

13.3.4. Records of storage and distribution of tissues and cells

Organisations undertaking storage of tissues or cells should keep the following records:

a. storage location and a transfer record if storage locations change;

b. date placed in storage;

c. date removed from storage;

d. records of storage temperature;

e. any incidents that occurred during storage.

In addition, when the tissues and cells are transported or distributed to hospitals or clinics for implantation, tissue establishments should keep the following records:

a. name of party responsible for distribution;

b. identification of the establishment, courier or individual who transported the tissues and cells at any stage between procurement and end use (implantation);

c. packaging records (e.g. records of the dry shipper used);

d. time and date of distribution of tissues and cells;

e. time and date of delivery of tissues and cells;

f. identification of the receiving establishment, clinician or end user/facility;

g. any incidents that occurred during distribution.

13.3.5. Records of end use of tissues and cells

The medical facility applying or implanting the tissues or cells to recipients should keep the following records:

a. identification of the supplier tissue establishment;

b. identification of the clinician or end user/facility;

c. type of tissues and cells;

d. product identification;

e. identification of the recipient;

f. date of implantation;

g. any incidents that occurred during implantation;

h. any adverse reactions in the recipient;

i. health outcomes of the ART child or ART children.

In the EU, the medical facility applying the tissues or cells to the recipients must keep the data stated above for 30 years. Some national standards require the end users to provide the supplying tissue establishment with the details of the patient in whom the tissues or cells were transplanted. Whether this information is sent to the tissue establishment or not, it is essential that the end user maintain these records because ultimately they are responsible for recording the fate of the tissues or cells.

In the case of ART, systems must be in place to follow-up children conceived after treatment. Such follow-up can be achieved only if a close working relationship exists between all stakeholders. That is, the tissue establishment, ART centre and parent(s) involved in the ART treatment.

13.4. Single European Code for tissues and cells

EU Tissue and Cell Directives (European Directives 2004/23/EC – Articles 8, 25 and 2006/86/EC – Article 10) set out the requirement to develop a single European coding system to identify and label tissue and cell products so as to support traceability of tissues and cells in the EU. The minimum set of information to be incorporated in the SEC was also set out in Annex VII of Directive 2006/86/EC, but without providing further details on its implementation. Consequently, new requirements which cover the full definition of the SEC, indications on its application, as well as obligations of the tissue establishments, Health Authorities and the European Commission have been adopted Directive 2015/565 and should be transposed into the national legislation by 29/10/2016.

Each tissue product will be assigned a specific code, which will identify and describe that product. It will be possible to decode the information to obtain text that describes the tissues or cells and their origin *via* an online code translator. In a multilingual world, codes are more useful than readable text because they are unambiguous. The coding system used to identify and label tissues and cells would be compatible with the current national and international system of

coding used in EU member states (Eurocode, ISBT128). In addition, a tissue and cell product coding system (European Code for Tissues and Cells [EUTC]) to be used by operators applying only local codes has also been proposed. This EUTC product coding system for tissues and cells developed by the EU comprises all types of tissues and cells with high-level terminology and their corresponding product codes.

13.4.1. Tools for implementation of the Single European Code

In 2011, the European Commission contracted external service providers to help implement the coding system. This consortium referred to as 'Eurocet 128' has developed the tools for implementation of the SEC [3]:

a. *EU Tissue establishment compendium* (register of all tissue establishments that are authorised, licensed, designated or accredited by the competent authorities of EU member states, and which also includes information on their location and authorisation);

b. *EU Tissue and cell product compendium* (register of all types of tissues and cells circulating in the EU and the respective product codes under the three permitted coding systems: EUTC, ISBT128 and Eurocode);

c. code-translator application.

These tools were transferred to the European Commission, who will be responsible for ensuring their maintenance and for updating the EU *Tissue and cell product compendium*, whereas updating the EU *Tissue establishment compendium* will be the responsibility of the EU Health Authorities.

These tools will be publicly available and free of charge. Hence, the EUTC used by EU member states may be also used by other interested countries.

13.4.2. Structure and format of the Single European Code

The code for each product will comprise two parts: a Donation Identification Sequence and a Product Identification Sequence (see Table 13.1).

Table 13.1. Single European Code for tissues and cells

Donation Identification Sequence			Product Identification Sequence			
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

13.4.2.1. *Donation Identification Sequence*

The coding system must identify each donation event because donors can potentially donate tissues and cells on several occasions (e.g. HSC 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 can, based on the donation identification system in place in their country, assign a unique number for the donation, which should be 13 characters in length. The unique donation number may be created locally, by the tissue establishment or centrally (provided by the Health Authority) or provided by an international organisation (e.g. the International Council for Commonality in Blood Banking Automation [ICCBBA]).

Hence, taken together, these codes will ensure that each donation will have a unique Donation Identification Number that can be used to label each tissue product.

13.4.2.2. *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 in ISO standard format (yyyymmdd). The product code includes an identifier of the coding system used ('E' for EUTC, 'A' for ISBT128 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, ISBT128, Eurocode) for which all tissues and cells 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, so a tissue and cell product code in the SEC can be 'translated' irrespective of the system used. EUTC provides only the basic nomenclature, but ISBT128 and Eurocode include more detailed product information (e.g. EUTC may represent a product type such as a tendon, whereas the other two systems specify whether the tendon is whole, shaped, or irradiated).

EU member states may decide to use only one product coding system (EUTC, ISBT128 or Eurocode) or two or all of them in parallel.

With respect to the format, the SEC on the label attached to each product will be readable by the eye, and proceeded by the acronym 'SEC' with the Donation Identification Sequence and Product Identification Sequence separated by a single space or as two successive lines.

13.4.3. Application of the Single European Code

Once the tissues and cells have been processed, the tissue establishment must label the product. For the tissues and cells distributed to organisations responsible for human application, the label should

include the full SEC (a Donation Identification Sequence and a Product Identification Sequence). For tissues transferred to other operators for further processing, with or without return (e.g. tissue establishments, third parties, manufacturers of advanced therapy medicinal products), as a minimum the Donation Identification Sequence should be applied on the accompanying documentation.

There are some 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. HSC), tissues and cells imported from non-EU countries into the EU in cases of an emergency and are authorised directly by the Health Authorities, and tissues and cells imported from non-EU countries into the EU that 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 national or international systems (e.g. ISBT 128, Eurocode) with a standard for barcoding and other forms of machine readability can continue using those systems while incorporating the requirements of the EU code. 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.

13.5. References

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3. Commission Directive (EU) 2015/565 of 8 April 2015 amending Directive 2006/86/EC as regards certain technical

requirements for the coding of human tissues and cells, available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2015.093.01.0043.01.ENG, accessed 27 May 2015.

Chapter 14

Biovigilance

14.1. Introduction

This chapter provides general guidance on implementation of good Vigilance and Surveillance (V&S) practice by all those involved in the processes of transplantation and assisted reproductive technology (ART) from donation, through banking, to clinical use and to regulation of the field. Tissue- and cell-specific chapters in Part B provide additional specific guidance on vigilance in those fields, particularly Chapter 25, which details several specificities within ART vigilance.

A programme of V&S is essential for ensuring quality and safety of tissues and cells for human application. The quality system focuses on preventing errors and maintaining a consistent standard of agreed specification for tissues and cells released for clinical application. However, occasionally, residual risks or procedural errors result in failures, disease transmissions or situations in which donors or patients are exposed to risk, even if not harmed. These occurrences can be classified into 'adverse events' (which refer to process failures that might lead to harm in a recipient or a living donor) and 'adverse reactions' (which refer to adverse outcomes that have indeed occurred with harm to a donor, a recipient, or a child born through *in vitro* fertilisation (IVF) with gamete donation). An adverse event may or

may not cause an adverse reaction. Similarly, an adverse reaction may or may not be related to an adverse event. Reporting of these incidents represents important learning opportunities that can help all procurement organisations, tissue establishments, cell therapy and ART facilities, and clinical users (and not just those involved in the incident in question) to improve their processes and to achieve higher levels of safety and quality [1, 2].

According to European Union (EU) definitions, a 'serious adverse event' (SAE) is 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 the patient or which might result in, or prolong, hospitalisation or morbidity. 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, incapacitating or which results in, or prolongs, hospitalisation or morbidity.

These definitions are reflected in the World Health Organization (WHO) Notify Library for V&S of Medical Products of Human Origin (MPHO), in which adverse outcomes are categorised as:

a. harm to a donor;

b. harm to a recipient;

c. harm to a foetus or offspring;

d. risk of harm.

In summary, an adverse reaction is an incident whereby a living donor, a recipient or a foetus or child created by IVF and intrauterine insemination (IUI) with donor gametes has been harmed, whereas an adverse event is an incident that results in a risk of harm, although no harm has yet occurred. All adverse reactions and adverse events should be documented and investigated by health professionals to ensure appropriate investigation as well as corrective and preventive

actions. 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 tissue establishment. Conversely, serious adverse reactions and events (SARE) are rare. Therefore, there are significant benefits associated with consolidating V&S data on regional, national or international scales.

If products containing tissues or cells are classified as advanced therapy medicinal products (ATMP) in the EU, the regulatory framework of pharmacovigilance must be applied. The relevant legal texts and guidelines are described on the Internet website for the European Commission [3]. For these products, donation, procurement and testing 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 ATMP are associated with adverse outcomes.

14.2. Management and quality

As for other vigilance systems, vigilance activities in the field of tissues and cells should be considered and recognised at all levels of tissue establishments that are authorised for tissue and cell activities, beginning with the strategic and senior management levels. The organisation of the vigilance system, as well as the role of the various parties involved, should be defined and broadly communicated within the tissue establishment.

Health Authorities are encouraged to draw up guidelines for vigilance systems, notification forms, and examples of SARE for tissues and cells that should be reported to them. Appropriate communication and co-ordination between procurement organisations, tissue establishments and centres carrying out clinical application are essential

for an efficient vigilance system. Any organisations or bodies involved in activities based on tissues and cells (including clinical users) should have standard operating procedures (SOP) in place that describe how to collect, report, investigate and communicate notifications for adverse reactions and events (ARE). The identification of a local co-ordinator, who has responsibility for V&S specified in his/her job description, is an effective measure. It is recommended that the QMS and the V&S system, both of which contribute to risk management policy, should be co-ordinated at the tissue establishment level according to guidelines established by the Health Authority and under the direct responsibility of the Responsible Person (RP).

Relevant SOP and management of collection and investigation of ARE data should be evaluated during the inspection process in the tissue establishment. Implementation of computerised and integrated systems for collection and management of ARE data is encouraged.

14.2.1. Non-serious adverse events and reactions

This chapter focuses on the detection, reporting and investigation of SARE. However, all ARE and non-compliances (including those with minor consequences) should be documented and reviewed regularly within the QMS of the tissue establishment. This allows trends to be monitored and actions to be taken to continuously improve quality and safety. One important role for the Health Authority is to define and inform tissue establishments and professionals of which adverse events and reactions should be notified to them through vigilance and which should be managed locally through the QMS of the tissue establishment.

14.2.2. Complaints

Complaints from any party (clinical users, donors, patients or third parties) should also be managed within the QMS. Formal acknowledgement should be sent immediately, and the complaint should be investigated promptly. Corrective actions should be communicated to the complainant (if appropriate). Each complaint should be considered

for classification as an SAE or SAR and should be managed as such if it meets the criteria described in this chapter.

14.3. Adverse reactions

Adverse reactions must be detected, reported, investigated and assessed in terms of severity, imputability, probability of recurrence or frequency and consequences. Efficient systems for rapid quarantine or recall of unsafe tissues or cells must be in place, along with procedures for look-back where donors or recipients are found to have been exposed to a risk. Important learning outcomes from each adverse reaction should be communicated to relevant stakeholders appropriately.

Several symptoms or situations suggest that an adverse reaction might have occurred in a recipient of a tissue or cell and should, therefore, be seen as 'triggers' for an adverse reaction report. Shown below are examples of reportable adverse reactions (abbreviated descriptions in square brackets):

a. suspected harm in living donor related to procurement [**Patient harm**];

b. unexpected[1] primary infections possibly transferred from the donor to the recipient (e.g. viral, bacterial, parasitic, fungal, prion) [**Infection from donor**];

c. 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, anaphylactoid reactions or anaphylaxis [**Hypersensitivity**];

1 In certain circumstances, clinicians may knowingly transplant an infective donation (e.g. a cytomegalovirus-positive bone marrow donation). In these circumstances, patients should be informed about benefits *versus* additional risks and there should be specific follow-up. Clinical and biological monitoring, as well as prophylactic or pre-emptive treatment, should comply with existing recommendations or regulatory requirements, where they exist.

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 from tissues and cells or associated materials [**Toxicity**];

h. unexpected immunological reactions due to tissue or cell mismatch [**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 donation [**Genetic abnormality**];

k. suspected transmission of other (non-infectious) illness [**Other transmission**];

l. transfusion associated circulatory overload in haematopoietic stem cell (HSC) transplantation [**Volume overload**];

m. neurological reaction [**Insult**];

n. severe febrile reaction [**Fever**];

o. other [**Other**].

14.3.1. 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 be 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 be alert to adverse outcomes and be aware when such outcomes might be associated with the tissues or cells transplanted;

d. physicians caring for children born after non-partner ART treatment who may detect a genetic abnormality and, by reporting it, prevent further distribution of sperm from that donor;

e. any other tissue establishment staff involved in any procurement and transplant activities;

f. other vigilance systems (e.g. material/device vigilance, pharmacovigilance, etc.), when issues of concern are detected that might impact on the safety of tissues or cells for transplantation.

V&S aims at improving patient safety, so consideration should be given to the possible role of patients and patient organisations in the notification process for adverse reactions.

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 outcome. Tissue establishments 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 might be prevented in the future.

For most types of well-established clinical applications of tissues and cells, detailed reporting of clinical outcome by the clinical user to the tissue establishment 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 tissue establishment might also be required for all highly-matched, life-saving transplants such as HSC 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.

Equally important is the need to detect 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 (see Chapters 21 and 25). Although long-term follow-up of living donors of tissues and cells is needed for some type of living donors (e.g. always for HSC donors but not for bone donors, it should be differentiated from vigilance. However, co-ordination between these activities should be encouraged.

14.3.2. Reporting adverse reactions

14.3.2.1. *Clinicians to tissue establishments*

Tissue establishments 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 tissue establishment that supplied the tissues or cells before investigation or confirmation. This approach will allow the tissue establishment to take appropriate precautionary actions to prevent harm to other patients, and to involve the tissue establishment in the investigation process. Clinical users should be encouraged to report all types of suspected adverse reactions (serious and non-serious) to the supplying tissue establishment to allow filtering of those considered to be serious and reportable to an authority at a later stage.

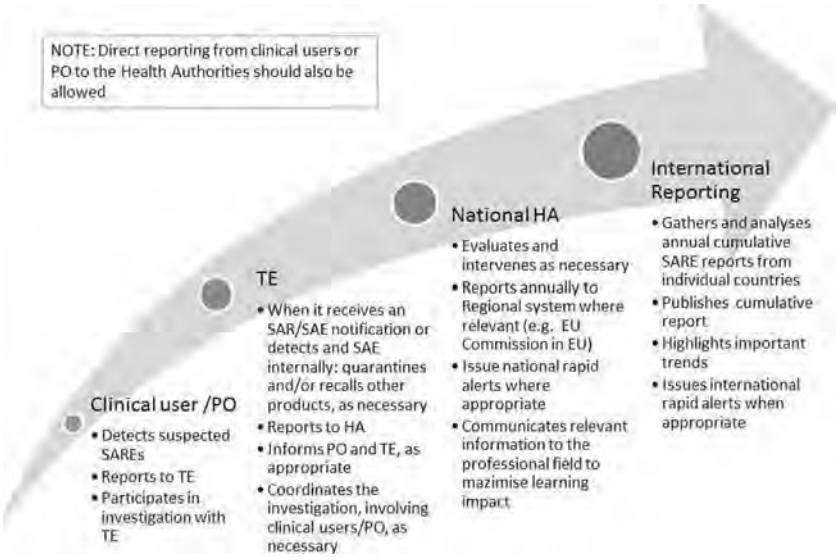
14.3.2.2. *Procurement organisations to tissue establishments*

Similarly, health professionals and procurement organisations should report adverse reactions in living donors to the tissue establishment, even if the adverse reaction is suspected to be only donation-derived, so that the broader implications for other centres and donors can be considered without delay.

14.3.2.3. *Reporting to regional/national programmes*

Information regarding SAR must be reported by tissue establishments to the Health Authorities so that the benefits of consolidated data can be realised and information about the lessons detected can be shared (see Figure 14.1).

Figure 14.1. Reporting flow for serious adverse events and serious adverse reactions



HA: Health Authority; EU: European Union; PO: Procurement Organisation; SAE: Serious Adverse Events; SAR: Serious Adverse Reactions; SARE: Serious Adverse Reactions and Events; TE: Tissue Establishment.

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 14.1 is the one used in the EU. It was proposed by the EU-funded project European Union Standards and Training for the Inspection of Tissue Establishments (EUSTITE) [4] for vigilance for tissues and cells and is based on the one used for haemovigilance. In the EU, all SAR that meet the descriptions of

'serious', 'life-threatening' or 'death' must be reported to the Health Authorities.

Table 14.1 Severity scale for serious adverse reactions

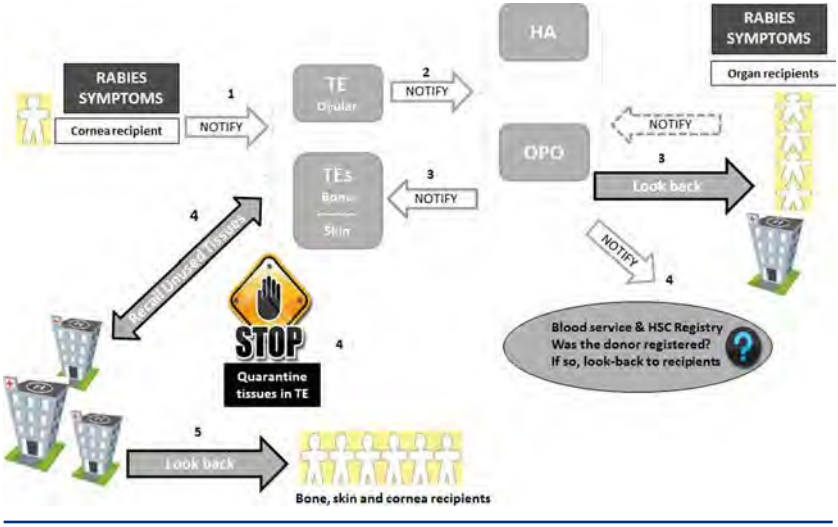
Not reportable	Insignificant	No harm to the recipient or living donor, and considered to be reportable as an event rather than a reaction according to EU Directives
	Non-serious	Mild clinical consequences that do not necessitate hospitalisation and/or result in long-term disability or consequences for 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 ART 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

The tissue establishment is responsible for providing clinical-user entities, procurement organisations and critical third parties with clear instructions, forms and guidance on how to notify adverse reactions in accordance with national or local requirements. Reporting and management of adverse reactions should be incorporated within the quality system of the tissue establishment with one or more SOP that describe the process for acknowledgment of notifications, investigation, follow-up on corrective and preventive actions and reporting to the Health Authorities if criteria are met. The procedures should enable rapid action to be taken by all affected organisations to protect

the safety of recipients. This may involve tissue and cell quarantine, recall and look-back in patients who have already had implicated tissues or cells applied. These actions may need to be taken by organisations other than the one that received the original notification. For example, the organ procurement organisation will play a central part where the donor was both an organ and tissue donor.

Figure 14.2 shows a series of actions that might need to be taken in a report of a suspected transmission from a deceased donor of organs and tissues. It highlights 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.

Figure 14.2. Example of an adverse reaction involving quarantine, recall and look-back



HA: Health Authority; HSC: Haematopoietic Stem Cells; OPO: Organ Procurement Organisation; TE: Tissue Establishment

Although SAR should, in general, be co-ordinated and centrally reported by tissue establishments 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 tissue establishment is not working correctly or where they do not have confidence, for whatever reason, that the report will be fully investigated.

14.3.2.4. *International reporting*

If SAR are detected in relation to tissues or cells that have entered international distribution channels, appropriate international collaboration should ensure that all of the stakeholders involved (clinicians, tissue establishments 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 they have received to the European Commission. Such international reporting allows for trend analyses on the basis of consolidated data. A published report from this programme is summarised in Appendix 9 of this Guide.

National Competent Authorities or Regulatory Agencies sometimes mandate SARE reporting for donors (as described above for the EU. However, HSC registries have implemented a global reporting system because products from HSC have specific characteristics:

a. Grafts are always collected from living voluntary donors who do not benefit from the donation itself. Thus, every effort must be directed at minimising donor adverse reactions.

b. Products from donors from HSC registries cross international boundaries more often than other products from tissues and cells (currently more than half of grafts donated by unrelated registry donors are used in countries different from their origin). Furthermore, most products are transported fresh and need to be infused <48 h after collection, thereby creating challenges for the management of transport.

c. SARE are rare and trends cannot be detected sufficiently at the donor centre, donor registry or at the national level.

The number of allogeneic HSC transplantations undertaken globally each year continues to increase. Therefore, there is a need for internationally standardised, continuous and rigorous follow-up of donors, not only from a legal, but also an ethical, viewpoint. Data on the incidence of short and long-term adverse reactions in donors are crucial to ensure maximum safety and availability of donors. This approach also forms the core of a global surveillance system that may identify rare (but serious) complications associated with donation. The World Marrow Donor Association (WMDA) has established a global surveillance reporting system (serious (product) events and reactions [S(P)EARs]) to collect, collate, analyse, distribute and react to serious adverse events/reactions (SAE/R) in unrelated HSC donors. This is done in collaboration with the WHO through sharing reported adverse incidents with the Notify Library.

Although reporting to the S(P)EAR system is voluntary, it is required of all HSC donor registries seeking WMDA accreditation. SARE reported to the WMDA are reviewed by the S(P)EAR Committee. A summary of reported S(P)EARs is issued on an annual basis. Particular attention should be given to vigilance of allogeneic related donors. They may be at higher risk as they tend to donate or be accepted as a donor with other criteria compared with unrelated donors (child donors [<18 years], older sibling donors [>45 years]). They also tend to take more risks (e.g. low iron storage, comorbidity) because they want to help a family member.

14.3.3. Investigation and assessment of adverse reactions

Adverse reactions in recipients of tissues or cells should be investigated by a team that includes the clinician that transplanted the tissues or cells, the tissue establishment 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 effective corrective actions. If relevant, experts in particular fields (e.g. viral transmission) should also be invited to participate in the investigation of the adverse reaction. The investigation should focus on establishing the level of imputability (i.e. the extent to which the

tissues or cells used clinically can be considered to have caused the adverse reaction). Where there is suspicion of an infectious disease transmission, investigation will rely heavily on the availability of an archived sample of donor serum. It is strongly recommended that frozen serum (and or cells or DNA) samples be maintained for every donor for vigilance investigation purposes. Consideration should also be given to keeping pre-transplant serum archives for transplant recipients to support imputability investigations. This approach is not advised for recipients of gametes or embryos.

The scale provided in Table 14.2, again developed by EUSTITE on the basis of the scale used in haemovigilance, and used in EU guidance to its member states, can be applied to describe the outcome of an imputability investigation. It is proposed that all adverse reactions be graded in terms of imputability. Table 14.2 also recommends specific approaches to the establishment of imputability for suspected infectious or malignant transmissions as proposed by Garzoni & Ison in the context of transplantation [5]. Imputability grades might change during an investigation and should, in general, be assigned at the point of initial notification and again at the completion of the adverse reaction investigation. The evaluation of imputability should be based on scientific or clinical data. The European Centres for Disease Control (ECDC), the WHO or other sources of epidemiological and risk information may be able to support the process.

Table 14.2. Scale describing the possible outcomes of an imputability investigation

	Adapted from EUSTITE-SOHO V&S [6]	Criteria for infectious and malignant transmissions adapted from the Disease Transmission Advisory Committee [7]
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	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 the tumour in a recipient *And it meets at least one of the following conditions:* • 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 that the same recipient was negative for the pathogen involved before transplantation

	Adapted from EUSTITE-SOHO V&S [6]	Criteria for infectious and malignant transmissions adapted from the Disease Transmission Advisory Committee [7]
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 that the same recipient was negative for the pathogen before transplantation

14.4. Adverse events

Adverse events can occur at any moment from donor selection to clinical application.

14.4.1. Detection of serious adverse events

For effective detection of adverse events, all relevant stakeholders must be aware of their responsibilities for identifying errors or unexpected results. This includes all staff in tissue establishments and procurement organisations, those working in organisations such as testing laboratories that provide 'third party' services to tissue establishments and clinical users who may also detect errors at the point of clinical use. In EU Directive 2006/86/EC, the definition of SAE includes those incidents often referred to as 'near misses', i.e. where an error or fault is detected and corrected without causing harm.

14.4.2. Serious adverse event reporting

Non-compliances with the quality system should be documented and investigated as part of the internal quality management system. On occasions, however, a particular non-compliance may be of such importance that it should be considered an SAE and reported through

the vigilance system. According to instructions from the European Commission to EU member states for annual vigilance reporting, deviations from SOP in tissue establishments (or other adverse events) that have implications for the quality and safety of tissues and cells should result in SAE reporting to the Health Authority if one or more of the following criteria [5] apply (see also Fig. 14.1, page 319):

a. inappropriate tissues or cells have been distributed for clinical use, even if not used;

b. the event could have implications for other patients or donors because of shared practices, services, supplies or donors;

c. the event resulted in a mix-up of gametes or embryos;

d. the event resulted in loss of any irreplaceable autologous tissues or cells or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;

e. the event resulted in the loss of a significant quantity of unmatched allogeneic tissues or cells.

14.4.3. Investigation and assessment of adverse events

Despite the fact that adverse events, by definition, have not, or not yet, involved harm to recipients or donors, the impact of an adverse event can be significant if considered in a broader way. The Impact Assessment tool given in Appendix 12 can also be applied to SAE to help reach a decision on the response required.

14.5. Vigilance co-ordination

Co-ordination between various systems of vigilance (e.g. medical device vigilance, pharmacovigilance) should be in place at the local level (tissue establishment) and at the Health Authority level.

14.5.1. 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 quar-

antine of tissues or cells. Rapid alerts should only be issued in exceptional circumstances. The following criteria have been identified in the SOHO V&S project [9] as triggers for rapid alerts within or between EU member states:

a. ARE of a serious or potentially serious nature;

b. potential risk to other individuals or other tissue establishments;

c. wider public health implications;

d. rapid intervention needed (preventive or corrective measures, urgent communication).

Within the EU, a system for Rapid Alerts (referred to as Rapid Alerts for Tissues and Cells [RATC]) is managed by the European Commission and enables the Competent Authorities of the EU member states to rapidly share urgent information regarding 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.

14.6. Vigilance communication

14.6.1. 'No blame' culture

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 health professionals is critical to support continued notification of AREs. All stakeholders, Health Authorities, 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 happen and that no programme of transplantation is risk-free. Programmes of training and awareness should be organised to encourage reporting. The message that reporting and disseminating V&S information can result in positive improvements for donors and patients should be promoted.

14.6.2. Vigilance experience and feedback

Health Authorities and professional societies should publish the results of their programmes without identifying individual centres, hospitals or individual people. Those tissue establishments or hospitals directly involved in specific incidents should also consider publishing their experience to alert others to the means by which they detected and confirmed the event or reaction.

The Notify Library is an initiative launched by the WHO and supported by the Italian National Transplant Centre (CNT) that has gathered information on documented types of adverse occurrences in transplantation and assisted reproduction. It has reviewed cases to identify general principles supporting detection and investigation. The database that has been constructed from the information gathered is accessible on a dedicated website [10, 11]. The database will be maintained and updated on this platform and is intended as a communication hub for institutions and organisations worldwide collaborating in the facilitation of access to V&S information to improve safety and efficacy.

14.7. Surveillance for new risks

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. 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 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 been recently mandated to provide risk assessment on particular epidemic agents, infectious diseases or new *in vitro* diagnostic techniques in the field of tissues and cells.

14.8. References

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Related documents:
Appendix 12 – Adverse reaction or event impact assessment tool;
Appendix 13 – Summary of data reported in the 2011 EU-wide report of serious adverse reactions and events associated with the clinical application of tissues and cells (data reported for 2010).

PART B. SPECIFIC REQUIREMENTS

Chapter 15

Ocular tissue

15.1. Introduction

Ocular tissues are obtained from deceased donors and can be prepared into corneal grafts, anterior and posterior lamellar grafts and scleral tissue. Corneal grafts are mainly used in diseases of the stroma (loss of transparency due to scars or loss of the normal curvature as in keratoconus) and of the endothelium (Fuchs endothelial dystrophy and re-interventions for endothelial decompensation of previous grafts). Lamellar transplant surgery has replaced full-thickness grafting in many cases; anterior lamellar grafts can be used for treatment of stromal scars or stromal distortion, whereas posterior lamellar grafts are applied for degenerative diseases of the corneal endothelium. For the past few years, eye banks have been providing precut lamellar corneal tissue for anterior lamellar keratoplasty (ALK), Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK). These services have saved precious time for corneal surgeons and reduced the risk of damage during corneal graft preparation in the operating room. Lamellar tissues for ALK and DSAEK may be prepared from the same cornea, thereby allowing treatment of two patients and increasing tissue availability.

Corneal allografts are carried out routinely without human leucocyte antigen (HLA) typing. However, certain conditions create high risks for immune rejection. For high-risk patients (vascularised cornea and previously rejected graft) HLA-matched corneas from tissue-typed donors may be beneficial.

The following generic chapters of this Guide (see Part A) all apply to ocular tissue banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);

b. Quality management, risk management and validation (Chapter 3);

c. Recruitment of potential donors, identification and consent (Chapter 3);

d. Donor testing (Chapter 5);

e. Distribution and import/export (Chapter 9);

f. Organisations responsible for human application (Chapter 10);

g. Computerised systems (Chapter 11);

h. Packaging and labelling (Chapter 12);

i. Traceability (Chapter 13);

j. Biovigilance (Chapter 14).

This chapter defines the additional specific requirements for ocular tissues.

Current use of limbal stem cells is described in Chapter 22. Autologous serum in the form of eye drops is used in ophthalmology. Their preparation, storage and use are described in Chapter 24.

15.2. Donor evaluation

15.2.1. Exclusion criteria for cornea donation

15.2.1.1. *General concepts*

There are some additional exclusion criteria for the donation of ocular tissues and some of the exclusions applied to other tissues are not applicable for cornea donation as described below.

15.2.1.2. *Donor age*

Provided that corneas are examined to exclude those with inadequate endothelium density, the donor age limits can be determined by the tissue establishment. However, the use of corneas from infants may be less suitable due to their high plasticity and high radius of curvature and will depend on surgical demand.

15.2.1.3. *Malignancies*

Donors with a history of or who are suffering from retinoblastoma, haematological malignancies (e.g. leukaemia, lymphoma, multiple myeloma, etc.) or malignant tumour of the anterior segment or the fundus of the eye must be excluded from donation of ocular tissues. Donors with a history of or suffering from other malignant diseases may be considered for cornea donation.

15.2.1.4. *Infections*

Persons with significant localised ocular infection (bacterial, viral, parasitic, mycotic) are excluded from donation of ocular tissues, including past ocular herpes infection.

Donors suffering from septic bacteraemia may be considered for cornea donation, provided that corneas are placed in organ culture media and tested with an appropriate microbiological procedure before transplant. In addition, a sample has to be tested for microbial contamination during storage in organ culture medium, applying a suitable method that allows the detection of potential bacterial or fungal contamination of the tissue. Donors colonised with multidrug-resistant bacteria should always be excluded.

15.2.1.5. *Eye diseases*

The following contraindicate cornea donation:

a. ocular inflammation (including known ocular involvement by systemic disease, e.g. sarcoidosis, rheumatoid arthritis);

b. corneal disorders including keratoconus, keratoglobus and dystrophy;

c. corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central area of the cornea.

15.2.1.6. *Previous intra-ocular or anterior segment surgery*

The following contraindicate cornea donation:

a. previous surgery that would prejudice graft outcome;

b. receipt of a corneal, sclera or limbal allograft;

c. refractive corneal surgical procedures, e.g. radial keratotomy, lamellar inserts, laser refractive surgery (photo-refractive keratectomy keratomileusis).

15.2.2. Exclusion criteria for other types of ocular tissue donation (e.g. sclera, limbal tissue, limbal cells)

a. No upper donor age limit needs to be set. The lower age limit is less certain. Use of ocular tissue from young donors will depend on surgical demand.

b. The donor evaluation criteria are the same as for general exclusion described in Chapter 4.

15.3. Procurement

15.3.1. *Post mortem* time

Ocular tissues should be procured as quickly as possible after cardiac arrest, preferably < 24 h, but some national and local standards allow procurement ≤48 h after cardiac arrest (a blood sample must still be taken <24 h of death).

15.3.2. Procurement team

Ocular procurement personnel must operate under aseptic conditions and must be appropriately clothed for the type of procurement to minimise the risk of contamination of the tissue to be removed and also of personnel. Usually, this requires hand disinfection, the wearing of sterile clothes and sterile gloves and the use of face masks or protective masks.

15.3.3. Procurement procedure

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 appropriate broad-spectrum antibiotic/anti-fungal solution may be used to further disinfect the eyes. Either the whole eye (enucleation by excision of extraocular muscles and the optical nerve) or the corneoscleral disc by *in situ* excision can be procured using aseptic procedures. It is recommended to procure the cornea with a sufficiently large rim of scleral tissue.

15.3.3.1. *Procurement of the whole eye*

After enucleation, the bulbus should be placed in a fixed position in a moist chamber. Broad-spectrum antibiotics may be used to further minimise bacterial contamination.

15.3.3.2. *Procurement of corneoscleral discs*

Peritomy should be undertaken to incise the conjunctiva in a circle as close to the limbus as possible, followed by sclerotomy ≥2–4 mm from the limbus. After excision, the corneo scleral disc should be immersed in an appropriate corneal storage solution.

15.3.3.3. *Procurement of scleral tissue*

Scleral tissue should be recovered from the whole eye after enucleation.

15.3.4. Reconstruction of the donor

An appropriate prosthesis or other material suitable for this purpose must be used to restore the appearance of the donor and the eye lids closed to restore the appearance of the donor.

15.4. Receipt of procured tissue at tissue establishments

Tissue should be transported to the tissue establishment as soon as possible after procurement or must be placed in the first processing solution directly after procurement. Whole eyes should be stored at 2–8 °C until processing is due to begin. Corneoscleral discs placed in a hypothermic storage solution after *in situ* excision should also be stored within these temperature limits. Corneoscleral discs should be stored at a dedicated temperature according to the used organ culture medium. The maximum storage time for eyes in a moist chamber is 48 h.

15.5. Processing and storage

15.5.1. Processing facilities

In selecting an appropriate air quality specification for ocular tissue processing, the criteria identified in Chapter 7 should be considered. Table 15.1 outlines factors to be considered for processing of ocular tissue.

Taking the factors detailed in Table 15.1 into consideration, when ocular tissues are exposed to the environment during processing, this should occur with particle counts and microbial colony counts equivalent to those of Grade A (European Union (EU) Good Manufacturing Practice (GMP) Guidelines) with an adequate background environment. For EU countries, the background must be at least Grade D (EU GMP). Taking the factors described in Table 15.1 into account, it is reasonable to apply at least this minimum standard for the processing of ocular tissue.

Table 15.1. Factors influencing the specification of processing air quality for ocular tissue

Criterion	Ocular tissue-specific
Risk of contamination of tissues or cells during processing	During processing, corneas are not exposed to the environment (closed system) or are exposed for a very short period (storage in organ culture). Then, the time of exposure is short and related to evaluation of endothelial cells or lamellar preparation.
Use of antimicrobials during processing	Cornealscleral discs may be stored in preservation media containing certain antibiotics as well as markers that change colour when micro-organisms grow in them. Organ culture storage methods allow the visual examination of the medium for microbial growth. Storage of corneas in organ culture allows the testing of samples of medium for microbial growth during storage.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling a piece of corneoscleral tissue when it is freshly preserved is feasible, but the amount of tissue available is very small. If it is preserved in organ culture media, the preservation solution must be submitted to testing and control of culture media.
Risk of transfer of contaminants at transplantation	Diseased or scarred corneal tissue is replaced by healthy corneal tissue, which is exposed to the environment, and thus is easy to treat topically.

15.5.2. Cornea processing methods

The following methods for preparation of the cornea are accepted:

a. excision of the corneoscleral disc from enucleated wholes eyes in the tissue establishment;

b. excision of the corneoscleral disc from the donor eyes *in situ*;

c. lamellar tissue preparation of the corneoscleral button obtained through one of the two ways mentioned previously, using manual or automated methods or lasers;

d. cryopreservation for long-term storage may be used for non-viable cornea tissue for tectonic grafts required for maintaining structural integrity of the eye.

15.5.3. Cornea storage methods

Corneal tissue must be viable when transplanted. The following storage methods are established in eye banking practice:

a. Hypothermic storage of the whole eye

A maximum storage time of 48 h is recommended, though this may be extended to 72 h for some purposes. Whole eyes in moist chambers should be stored at 2–8 °C.

b. Hypothermic storage of the corneoscleral disc in a corneal storage solution

The maximum storage time at 1–10 °C depends on the storage medium used (usually 7 days, see the manufacturer's instructions for the medium in use). It is recommended not to exceed the prescribed storage time. An inspection of the endothelium is mandatory and cell loss during storage must be taken into account, except for tissue designated for emergency or anterior lamellar grafting. Handling instructions for preparation of the tissue for surgery should be included. Testing of the hypothermic storage medium and/or remnants of sclera at the time of surgery is at the discretion of the surgeon or may be recommended by the tissue establishment.

c. Storage of the corneoscleral button by organ/tissue culture

It is recommended to keep the storage time as short as possible, with a maximum storage period of 5 weeks at 28–37 °C. It is at the discretion of the Responsible Person (RP)/Medical Director to prolong storage time, provided that documentation (evidence or validation of the procedure) is present to support this. An inspection of the endothelium is mandatory at the end of the storage period, except for tissue designated for emergency or anterior lamellar grafting.

Renewal of the storage medium using aseptic procedures during the storage period is at the discretion of the RP/Medical Director, and

is dependent upon the recommendations of the manufacturer of the storage medium in use.

To reverse the stromal oedema that occurs during organ culture, the cornea should be transferred to a solution containing a macromolecule to increase osmolality (i.e. 'transport solution' or 'deswelling solution'). The cornea may be kept at 18–37 °C for ≤5–6 days, depending on the medium used.

d. Storage of lamellar corneal tissue

Lamellar corneal tissue prepared from corneoscleral discs stored at 1–10 °C can be stored ≤2–3 days before transplantation, taking into account that the maximum prescribed storage time would not be exceeded.

Corneascleral discs stored by the organ culture method must be stored in a deswelling solution for ≥24 h before preparing lamellar corneal tissues for DSAEK. Then, the posterior lamella positioned on the anterior stromal cap can be stored in a deswelling solution for ≤1 week [1].

Descemet endothelial grafts are dissected directly from organ-cultured corneascleral discs and can be stored in the organ culture solution for an additional week before transplantation with a moderate decrease in endothelial cell density [2, 3].

15.5.4. Sclera processing and storage

After removal of the corneoscleral disc from the eye, the sclera is prepared using aseptic techniques by removing the remaining contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). The tissue can also be cut into pieces.

Sclera can be stored (whole or in separately packed pieces) at ambient temperature (in ≥70% (*v/v*) ethanol, glycerine, fixed in formalin or freeze-dried), frozen or kept in a refrigerator at 2–8 °C (in a hypothermic storage solution, 70% ethanol or saline with antibiotics). However, sclera can only be kept for a short period (≤7 days) in hypothermic solutions or in saline with antibiotics in the refrigerator.

15.6. Microbiological testing of the storage medium

a. Organ culture storage method for corneas

Microbiological testing of storage media samples is mandatory. A minimum storage period ≥3 days is mandatory before taking samples for microbiological testing (see Chapter 8).

A second microbiological test is mandatory at the time of terminal evaluation and transfer of the cornea into the transport medium.

In addition to microbiological testing, the culture medium should be inspected regularly for cloudiness, which may indicate microbial contamination.

b. Hypothermic storage method for corneas

Due to the short time of storage, it is not possible to wait for the final result of sensitive microbiological testing of the culture medium. However, sampling of the culture medium one day after the start of the storage period, or just before delivery for clinical use, is recommended. The treating physician should be informed of a positive result as quickly as possible. Also, a recommendation to the surgeon to undertake microbiological testing on the cornea storage medium or on remaining scleral rim at the time of surgery is recommended.

c. Sclera

Microbiological testing must be carried out after processing.

15.7. Quality control and cornea evaluation

Quality control tests on corneal grafts should consider at least the following minimum quality criteria:

a. no evidence of microbiological growth (aerobic or anaerobic bacteria, yeast or fungi);

b. endothelial characteristics (density and viability of cells);

c. morphology and integrity of the cornea layers;

d. diameter of clear central area of cornea.

Depending on the specific use of the cornea, it is necessary to check and document the conditions of the:

a. epithelium (if intended for a full-thickness graft, superficial or deep anterior lamellar graft, or a limbal graft), taking into account that the epithelium may detach during storage;

b. corneal stroma (if intended for a full-thickness graft or a superficial or deep anterior lamellar graft). There should be no central opacities or scars. The stroma of organ cultured corneas may be hazy but should be transparent after reversal of stromal oedema in transport medium;

c. endothelium, which is essential for maintaining corneal transparency (if intended for a full-thickness graft or a posterior lamellar graft).

The quality control tests to be carried out are:

a. Macroscopic inspection

Without optical instruments, the donor eye must be inspected *in situ* for corneal transparency and corneal disease, such as:

- abnormalities of the external globe;
- signs of previous surgery of the anterior segment;
- epithelial abrasions, retention of excessive orbital tissue or laceration of the globe;
- epithelial defects;
- stromal opacities. A mild arcus senilis with a defined clear central zone may be acceptable. The minimal diameter of the clear zone is at the discretion of the RP/Medical Director;
- abnormal corneal shape (keratoconus, micro- or megalocornea);
- condition of the anterior chamber (shape, evidence of blood);
- abnormalities, such as the pterygium extending to the optical zone extending over the optical zone of the cornea.

b. Slit lamp evaluation

Slit lamp examination (before decontamination and dissection) is highly recommended, but not mandatory. It facilitates exclusion of pathological changes of the epithelia or stroma (e.g. scars, oedema, significant arcus, striae, epithelial defects, endothelial guttae or disease, polymegethism, pleomorphism, infiltrates or foreign bodies).

c. Microscopic evaluation of the endothelial cells of the cornea

Examination by one of the following methods is necessary:

i. Specular microscopy

The appearance of the endothelial cells with specular microscopy varies with temperature, type and time of preservation and media used. Evaluation of corneas at ambient temperature is recommended.

ii. Transmitted light microscopy (bright field, phase-contrast).

For ease of cell counting, induction of cell swelling with a hypotonic solution (e.g. sucrose 1.8% for a few seconds or hypotonic buffered salt solution (BSS) for 4 min) or with saline (0.9% for 4 min) is necessary to make the boundaries of endothelial cells visible. The exposure time to these solutions must be limited. Use of a vital stain (e.g. 0.06–0.4% trypan blue) may help to identify dead cells and denuded Descemet membrane. Cell counting should be done at different areas, centrally and paracentrally ≤5–6 mm from the centre.

A density of endothelial cells of <2,000 cells/mm^2, moderate-to-severe signs of polymegethism and pleomorphism, signs of significant cell loss during organ/tissue culture (>20% at the end of the first-phase storage period) or dead cells (scattered single dead cells would be acceptable, whereas larger areas of dead cells are not) are, in general, considered as contraindications for long-term survival of grafts.

15.8. Examples of serious adverse reactions and serious adverse events

The Notify Library includes some well-documented cases of adverse reactions and adverse events in transplantation of ocular tissue, as shown below.

- A case of donor-to-recipient transmission of the herpes simplex virus (HSV) by cornea transplantation was confirmed by polymerase chain reaction-based DNA fingerprinting of donor and recipient HSV strains is described (Record Number 429);
- In Record Number 20, a transplant-acquired diagnosis of rabies is supported by temporal association of the recipient's illness, lack of other exposure to rabies, and the retro-orbital pain of the recipient of the corneal transplant;
- In Record Number 338, a case of transmission of T-cell lymphoma is described, whereby molecular analyses were used to detect the same alleles in HLA-DQα testing of the recipient and donor of the graft;
- Record Number 720 describes the first reported case of donor ocular tissue being examined and then shipped to the eye bank with a contact lens on the cornea, thereby highlighting poor *in situ* inspection before recovery (or an inadequate recovery method).

For further cases of adverse outcomes associated with banking of ocular tissue, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by the substance type, type of adverse occurrence and by Record Number.

15.9. References

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Chapter 16

Amniotic membrane

16.1. Introduction

Human amniotic membrane (hAM) is the innermost, semi-transparent layer of the foetal membranes formed by a single layer of cuboidal, epidermis-like cells that are attached to a thick basement membrane and an avascular stromal matrix consisting of scattered fibroblasts in a collagen scaffold.

It has some unique properties. Clinical and experimental data have shown [1–5] that hAM facilitates the proliferation and differentiation of epithelial cells, maintains the original epithelial phenotype, promotes differentiation of goblet cells, reduces scarring and vascularisation, and lacks immunogenicity. Collagen type I, III, IV, V and VII, laminin and fibronectin have been identified in the amniotic basement membrane and stroma. 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 proteinase activity and decreased lipid peroxidation. In addition, it has anti-adhesive and anti-bacterial activities, along with the ability to modulate stromal scarring. It also protects wounds and reduces pain. Moreover, hAM epithelium produces various growth factors (e.g. epithelial growth

factor, basic fibroblast growth factor, hepatocyte growth factor, keratocyte growth factor and tumour growth factors) and cytokines (e.g. interleukin 6 and 8). These characteristics have led to the use of hAM in clinical practice; mainly in the treatment of ophthalmology, but also to encourage epithelialisation in burns, as a temporary or long-term wound dressing, as graft material over skin ulcers to replace mucosa, in arthroplasty and intra-abdominal and reconstructive surgery.

The following generic chapters of this Guide (see Part A) apply to hAM banking and must be read in conjunction with this chapter:

a. Introduction (Chapter 1);
b. Quality management (Chapter 3);
c. Donor evaluation (Chapter 4);
d. Donor Testing (Chapter 5);
e. Procurement (Chapter 6);
f. Processing and storage (Chapter 7);
g. Principles of microbiological testing (Chapter 8);
h. Distribution and import/export (Chapter 9);
i. Organisations responsible for human application (Chapter 10);
j. Computerised systems (Chapter 11);
k. Packaging and labelling (Chapter 12);
l. Traceability (Chapter 13);
m. Biovigilance (Chapter 14).

This chapter defines the additional specific requirements for hAM.

16.2. Donor evaluation

Amniotic membrane could be contaminated by normal vaginal flora during normal vaginal delivery. Therefore, in general, it should be obtained under aseptic conditions after elective caesarean section after a full-term pregnancy. If the hAM and was obtained during vaginal

delivery, different sterilisation procedures should be applied (e.g. sterilisation by gamma irradiation).

16.2.1. Specific exclusion criteria

Diseases of the female genital tract or other diseases of the donor or unborn child that might present a risk to the recipient include:

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. meconeum ileus.

Individual tissue establishments may have additional exclusionary criteria.

16.3. Procurement

16.3.1. Procurement facility and procurement team

Placenta and/or procured foetal membranes are collected from living donors by medical staff at a gynaecological clinic after caesarean section or vaginal delivery. Staff undertaking procurement must be dressed appropriately for the procedure so as to minimise the risk of contamination of the tissue to be procured and any hazard to themselves.

16.3.2. Storage and transport after procurement

Placenta and/or procured foetal membranes should be stored at appropriate temperatures to maintain their characteristics and biological functions.

The storage and transport time of procured foetal membranes should be kept as short as possible (the recommended maximum time is 24 h) and a temperature of 2–10 °C should not be exceeded. If the foetal

membranes are prepared <2 h after the delivery, the placenta may be transported at room temperature.

The placenta and/or procured foetal membranes should be placed in a sterile receptacle containing a suitable transport medium if necessary (if transport time >2 h). The sterile packaging should then be placed inside an adequately labelled sterile container to be transported to the tissue bank. Individual tissue establishments should validate the composition of the transport medium and determine if an antibiotic cocktail is required.

The temperature during transport to the tissue establishment must be maintained. The stability and reliability of temperature should be guaranteed by the container or mode of transport used and time interval. In cases of unexpectedly high or low environmental temperatures, a temperature-recording unit (data logger) should be enclosed in the container that records temperature in, at least, half-hourly intervals unless the transport system has been previously validated to maintain the required temperature for a defined period of time.

16.4. Processing and storage

16.4.1. Receipt of procured placenta at the tissue establishment

The procured foetal membranes can either be:

a. stored at 2–10°C (e.g. in the refrigerator). The refrigerator temperature should be monitored and permanently recorded. In this case, processing should be carried out ≤48 h after procurement. If the process is intended to maintain amnion cell viability, then it is recommended that the cell nutrient medium be changed in a climate-controlled environment on receipt of the donated material;

b. stored at a temperature lower than –60 °C (ultra-low-temperature freezer).

16.4.2. Processing facilities

In selecting an appropriate air quality specification for hAM processing, the criteria identified in Chapter 7 should be considered. Table 16.1 outlines factors to be considered for hAM processing.

Table 16.1. Factors influencing the specification of processing air quality for hAM

Criterion	Amnion-specific
Risk of contamination of tissues or cells during processing	During processing, amniotic membranes are necessarily exposed to the processing environment for extended periods during dissection, sizing and evaluation of their characteristics.
Use of antimicrobials during processing	Soaking in antibiotics is an antimicrobial step that can be used when processing amniotic membranes. It is important to validate the antibiotic solution and to list the micro-organisms that are acceptable pre-decontamination. Glycerolised, lyophilised, and frozen amniotic membranes can be exposed to sterilisation processes, so the processing environment may not be as critical for those amniotic membrane types. 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 amniotic membrane for microbiological analysis following antibiotic soaking is not extensive; typically a very small percentage is sampled. However, the storage medium can also be sampled.
Risk of transfer of contaminants at transplantation	Although not vascularised, amniotic membrane can support microbiological contaminants and has transmitted bacterial and viral agents. Amniotic membrane is mostly used for ophthalmologic purposes. However, amniotic membranes are used for other indications, such as burns, skin ulcers, and arthroplasty. Intra-abdominal and reconstructive surgery using amniotic membranes has also been described. These patients can also develop immunosuppression by various mechanisms and, despite recent advances in therapy, have a high risk of death from infections.

Taking the factors from Table 16.1 into consideration, it is appropriate that processing of hAM allografts should take place in a bacteriological and climate-controlled environment (including control of temperature, humidity, ventilation and air filtration) with validated cleaning and disinfection. For cryopreserved hAM where less bacterial inactivation is possible, a particularly clean and well-controlled environment is required.

Many national requirements allow processing of tissues without subsequent microbial inactivation (including hAM) only in air quality equivalent to those of Grade A as defined in European Union (EU) Good Manufacturing Practice (GMP) with Grade C or B as a background, as appropriate for processing tissues prone to tissue contamination due to extensive manipulation or processing phases at ambient temperature and which are not followed by a terminal sterilisation step. Within the EU, tissues exposed to the environment without subsequent microbial inactivation should be processed in environments with an air-quality equivalent to those of Grade A, as defined in EU GMP, with a background environment at least equivalent to Grade D.

16.4.3. Processing methods

Procured hAM can be processed to facilitate longer storage periods until transplantation in suitable patients. The methods used should be up-to-date and validated. Tissue establishments tend to use different graft processes, according to their own standard operating procedures (SOP) and mandatory regulations.

The foetal membranes should be rinsed several times, and the amnion and chorion should be mechanically separated and blood residues should be removed according to a documented standard operating protocol. The amnion should be placed separately on a suitable carrier membrane (e.g. nitrocellulose) if it needs to be divided into smaller pieces.

Maximum storage durations should be specified in all cases. There are several methods of hAM preparation and preservation (see below).

16.4.3.1. *Cryopreservation*

Amniotic membrane can be cryopreserved in a medium with cryoprotectant using an appropriate container (bags or cryovials) and transferred to liquid nitrogen tanks (vapour phase, below –130 °C).

However, if the hAM is stored in sterile glycerol medium, the storage temperature is usually below –60 °C.

If procurement or processing methods were not done under sterile conditions the tissue, after processing, should be (after packaging) sterilised with gamma irradiation or accelerated electron beams. The dose of irradiation as the inactivation process should be validated.

If stored at higher temperatures, the process should be validated, taking into account the quality of the tissues over time.

16.4.3.2. *Heat-dried amniotic membrane*

The tissue is dried overnight in an oven at 40±2 °C, then radiation-sterilised. The membrane loses many of its biologic properties due to the high temperature, so hAM preserved in this way is typically used as a biologic dressing for the management of burns.

16.4.3.3. *Air-dried amniotic membrane*

After the amniotic membrane is separated and washed in sterile conditions, it is air-dried overnight in a laminar flow hood. It can then be packaged and radiation-sterilised. Although high temperatures are not applied using this method, some properties of the amnion are lost or altered due to dehydration. Amniotic membrane prepared in this way can be used for wound dressings. Air-dried amniotic membranes should be transported at ambient temperature.

16.4.3.4. *Lyophilised (freeze-dried) amniotic membrane*

The amniotic membrane can be cut into pieces and rapidly frozen at –50 °C to –80 °C. Then it is vacuum-dried using a freeze-drying device. Water in the tissue is extracted through sublimation until a final water content of 5–10% is attained. The tissue can then be packaged and radiation-sterilised. This type of preparation induces

minimal changes in the properties of the amniotic membrane and the product can be stored at ambient temperature. Lyophilised amniotic membranes should be transported at ambient temperature. This type of preparation is mainly used for the management of wounds.

16.4.3.5. *Preservation in cold glycerol*

Glycerol has long been used as a cryoprotective agent. Due to its high osmotic potential, it can extract interstitial water from the amniotic membrane. Typically, 80% glycerol is used to store the amniotic membrane, which can then be preserved at 2–8 °C for a long time, although it loses some of its biologic properties. Amniotic membranes preserved in this way are used as dressings for burns.

16.4.3.6. *Antibiotic impregnated amniotic membrane*

After separation, the amniotic membrane is placed overnight in an antibiotic solution composed of different types of wide-spectrum antibiotics and an anti-fungal agent and then frozen at −80 °C. The resultant amniotic membrane is suitable for the management of infected wounds by providing an appropriate concentration of antibiotics to the wound surface.

16.5. Quality control

During procurement and preservation of hAM, reliable macroscopic examination of the donor placenta should be undertaken to exclude visible pathological changes.

Samples for detecting aerobic and anaerobic bacteria and fungal cultures should be obtained from the transport/storage medium, or the rinsate from washings of the membrane, and from pieces of the membrane, before placing it in antibiotic solutions and before packaging. Microbiological controls for the detection of bacteria and fungi should be carried out according to those described in Chapter 8.

16.6. Distribution

For cryopreserved hAM, distribution temperatures between −60 °C and −85 °C should be maintained, for example, using dry ice. Trans-

port temperatures of cryopreserved hAM above –60 °C are to be strictly avoided to ensure the stability of the product and maximum safety for the recipient. The tissue establishment must ensure that all storage processes are undertaken under controlled conditions.

Lyophilised, air-dried and freeze-dried amniotic membranes can be stored and distributed at ambient temperature. Foetal membranes preserved in cold glycerol should be transported under cold temperatures at 2–10 °C.

16.7. References

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Chapter 17

Skin

17.1. Introduction

Donor skin grafts can have critical roles in the treatment of severely burned patients by acting as a temporary skin replacement, vehicle for dermal tissue to guide repair in a more physiological manner, a means of reducing scarring, maintaining homoeostasis within the body, and controlling pain. For these reasons, it is considered the treatment of choice for substantial loss of skin tissue, such as in cases of severe burns for which it acts as a life-saving therapy. Homologous skin is also considered to be an excellent biological dressing for the treatment of other types of skin loss such as venous ulcers, decubitus ulcers, toxic epidermal necrolysis (Lyell's syndrome), surgical wounds and congenital epidermolytic skin disorders. In these cases, skin grafts promote re-epithelisation, formation of granulation tissue (small vessels in the wound bed), shorten healing time, control pain, and protect important structures (e.g. tendons, bones, cartilage, nerves).

These factors explain the constant demand for skin allografts by burns centres, and reconstructive surgery units, where the capacity of these bio-products to 'take' and integrate into the wound bed are exploited.

The shortage of homologous 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 last 30 years, a huge number of biological 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.

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, risk management and validation (Chapter 2);

c. Recruitment of potential donors, identification and consent (Chapter 3);

d. Donor Testing (Chapter 5);

e. Procurement (Chapter 6);

f. Processing and storage (Chapter 6);

g. Principles of microbiological testing (Chapter 8);

h. Distribution and import/export (Chapter 9);

i. Organisations responsible for human application (Chapter 10);

j. Computerised systems (Chapter 11);

k. Packaging and labeling (Chapter 12);

l. Traceability (Chapter 13);

m. Biovigilance (Chapter 14).

This chapter defines the additional specific requirements for skin.

17.2. Skin-specific donor evaluation

17.2.1. Skin inspection

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 checked for multiple or dysplastic naevi, dermatitis, local infections, ectoparasites or rashes. The results must be recorded and taken into account before initiating the final release of the tissue.

17.2.2. Skin-specific exclusion criteria

In addition to the donor exclusion criteria described in Chapter 4, there are some specific conditions that exclude skin donation. 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 the skin for optimal functional results. The following conditions contraindicate skin donation:

a. auto-immune dermatoses;

b. systemic connective tissue diseases;

c. diseases affecting the dermis (dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria);

d. mechanical or microbial damage to the skin;

e. burns at the location on the body where skin is to be procured;

f. toxicity of the skin as a result of the presence of toxic agents or poisons;

g. presence of possible melanomas;

h. systemic infections, which at the time of donation are not under control.

The following relative contraindications for skin donation should be considered on a case-by-case basis:

i. extensive lacerations or scars;

j. skin diseases with extensive involvement, such as psoriasis, eczema or nodules;

k. relevant ulcers, decubitus, pyoderma or mycoses;

l. skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos);

m. diabetes mellitus with skin complications (e.g. ulcers).

The common practice is not to procure skin from donors aged <15 years but there is no any upper limit for age.

17.2.3. Skin-specific procurement procedures

Although skin can be obtained from multiple-organ donors it is obvious that refrigeration of the body before procurement will reduce skin contamination and facilitate skin procurement due to the harder consistency of the subcutaneous tissue. Procurement from living donors can also be carried out in patients undergoing abdominoplasty who consent to tissue donation.

Skin from living donors having abdominoplasty procedures is procured to obtain full-thickness skin grafts (1–3 mm). The area is prepared by depilation and disinfection.

17.2.4. Skin-specific *post mortem* time

It is recommended to procure the skin within 24 h of death if the body has been cooled or refrigerated within 6 h of death. It may be possible to extend procurement times ≤48 h if skin processing has been validated to guarantee quality and microbiological safety. If the donor body was not refrigerated after death, then procurement should be completed within 12 h of death.

17.3. Skin procurement

17.3.1. Skin-specific 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 pre-defined 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. Procurement teams should follow an effective and validated donor disinfection procedure, which can significantly reduce the microbial positivity rate of processed skin samples. Studies show that whether the skin procurement is done before or after bone procurement, the contamination rate of skin is no different if the procurement process is controlled and standardised [1].

17.3.2. Skin procurement procedure

The skin is procured under aseptic conditions after adequate shaving of the donor areas and appropriate preoperative disinfection of the skin to remove the transient and reduce the resident microbial flora. A standardised procedure should be established for skin disinfection by the tissue organisation and allocated to all procurement sites.

The procedure should reduce the bioburden. Therefore, suitable disinfectants, such as povidone iodine or chlorhexidine, should be chosen. Their concentrations and the durations of exposure should also be evaluated.

A local sterile field using sterile drapes must be used prior to procurement to effectively reduce microbial contamination. Skin grafts are cut by electric or battery-operated dermatomes from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs. Graft thickness is usually 200–800 microns and each graft should be cut as long and homogenously as possible

17.3.3. Reconstruction of the skin donor

For aesthetic reasons and with a view to a respectful reconstruction of the donor, it is not acceptable to take skin from the neck, face and other typically exposed areas of the body that might be visible when people pay their last respects to the donor. Once the tissue has been procured, appropriate garments or sealing agents (polymers) to

prevent leaking and oozing from sites where tissue has been obtained should be used.

17.3.4. Procurement documentation

The organisation responsible for the skin procurement must produce a procurement report to be provided to the tissue establishment. In addition to the generic requirements defined in Chapter 6, it must contain a description and identification of the procured skin (including samples for testing).

17.3.5. Skin transportation to the tissue establishment

Procured skin must be packaged immediately after procurement in a suitable transport medium in sterile, sealed, refrigerated containers that are labelled adequately to ensure traceability (see Chapter 6). Storage at low temperatures prevents proliferation of most bacteria and fungi and maintains viability. The package must be cooled during transport to the tissue establishment. If skin grafts are to be processed for glycerolisation, refrigerated transportation is not required and the skin can be placed, after recovery, in 50% glycerol solution at ambient temperature.

17.3.6. Receipt of procured skin at the tissue establishment

The procured skin should be transferred to the tissue establishment as soon as possible after procurement or should be put in an initial processing fluid directly after procurement. After the skin has been received by the tissue establishment, processing of the skin should commence within 72 h of procurement having taken place. Before processing, the skin should be kept in a refrigerator, in a physiological medium with sufficient buffering capacity. It is recommended that the cell nutrient medium used for viable grafts be changed in a climate-controlled environment shortly after receipt of skin grafts.

17.4. Skin processing

The recovered skin is processed to allow longer storage periods until transplantation in suitable patients. The methods used must be in line

with current state-of-the-art and validated procedures (see Chapter 7). Different tissue establishments apply specific preparation processes according to their own standard operating procedures (SOP) and any applicable local authorisations.

Table 17.1. Factors influencing the specification of processing air quality for 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 antimicrobial step possible for cryopreserved skin. This is why it is indispensable to process tissues in clean rooms, where rigorous limits on contamination by particles and microbes are observed. Glycerolised skin or lyophilised skin can be exposed to more robust decontamination or sterilisation processes, so the processing environment is not as critical for those skin types. Glycerol (85%) itself has been shown to inactivate micro-organisms.
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. Sterility testing has significant statistical limitations, and can be used to detect only large contamination. Final sterility testing may even be unreliable, especially if antibiotics remain on tissues [2].
Risk of transfer of contaminants at transplantation	Although not vascularised, cryopreserved skin can support microbiological contaminants and has transmitted bacterial and viral agents. 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 immunosuppression by various mechanisms and, despite recent advances in therapy, have a significant risk of death from infection.

17.4.1. Processing facilities

In selecting an appropriate air quality specification for skin processing, the criteria identified in Chapter 7 should be considered. Table 17.1 outlines the factors to be considered for skin processing.

Taking the factors detailed in Table 17.1 into consideration, it is appropriate that processing of skin allografts takes place in a bacteriological- and climate-controlled environment (including control of temperature, humidity, ventilation and air filtration), with validated cleaning and disinfection to ensure the best aseptic conditions that should be standardised, monitored and maintained constantly. A particularly clean and well-controlled environment is required for cryopreserved skin: a Grade A environment with Grade B background is recommended to ensure aseptic processing of cryopreserved skin allografts.

Within the European Union (EU), tissues that are exposed to the environment without a subsequent microbial inactivation process (e.g. cryopreserved viable skin) should be processed in environments with an air quality equivalent to those of Grade A, as defined in EU Good Manufacturing Practice (GMP), with a background environment at least equivalent to EU GMP Grade D. Many national requirements for skin processing environments are more stringent, requiring EU GMP Grade B or C as a background, which might be more appropriate for processing tissues prone to tissue contamination due to extensive manipulation or processing phases at ambient temperature and that are not followed by a terminal sterilisation step.

17.4.2. Skin decontamination and preservation

After being received by the tissue establishment, skin is decontaminated and processed into, glycerol-preserved, cryopreserved, decellularised or lyophilised skin allografts according to the procedures of the tissue establishment. Skin grafts to be cryopreserved are processed immediately after receipt in order to maintain cell viability. Skin allografts can also be processed as de-epidermised dermis and acellular dermis. All human tissues intended for transplantation are

processed into specimens appropriate for clinical use. Processing must not change the physical properties of the tissue so as to make it unacceptable for clinical use. All processes must be validated in accordance with the guidance given in Chapter 2. Compliance of processing standards with published guidelines ensures adherence to generally accepted quality standards.

17.4.3. Skin graft sizing

Skin allografts can be cut into specific sizes or can be cut to the actual size required for the skin grafts. Skin grafts may be provided as meshed or non-meshed grafts. The graft's rough edges should be trimmed and, typically, a rectangular shape is measured with a ruler or callipers. The linear dimensions and area of each skin graft must be recorded. The grafts should then be packaged in appropriate sterile packages.

17.4.4. Glycerol-preserved skin allografts

Glycerol-preserved skin allografts were developed [3] to maintain skin allografts without freezing using an increasing series of glycerol concentrations (50%, 70%, 85%). Glycerol-preserved allografts (GPAs) are de-vitalised skin grafts considered to be safe products due to the antibacterial/antiviral properties of high concentrations of glycerol [4–6]. The glycerol solutions used should be sterile and of high quality (e.g. see *European Pharmacopoeia* monograph Glycerol 85 per cent [0497]). Glycerol-preserved skin allografts can be stored at 2–8 °C for several years.

17.4.5. Fresh skin allografts

The use of fresh skin allografts is not the preferred option as it may not allow to complete donor screening and extensive microbiological testing.

However, some tissue establishments use fresh skin allografts as it is possible to maintain their structural integrity and cell viability for short periods of time (maximum, 7–8 days) [7]. Fresh,

refrigerator-stored skin allografts were initially preferred in Burns Units due to their high cell viability [8].

17.4.6. Cryopreserved skin allografts

Cryopreservation of skin allografts necessitates rapid processing phases at low temperatures in order to maintain cell viability. Inappropriate storage compromises the potential to restore normal metabolic activity and, thus, physiological functioning after transplantation.

Cryoprotectants such as glycerol or dimethyl sulfoxide (DMSO) can adversely affect cell viability. A controlled and validated refrigeration procedure is recommended to preserve cell viability. Cell viability should be validated by qualitative (histomorphology and/or immunochemistry) and quantitative methods. Cryopreservation between −60 °C and −80 °C is a method for medium-term (months) preservation of viable skin allografts. Viable skin allografts can be stored in liquid or vapour nitrogen for longer periods (years).

17.4.7. Lyophilised skin allografts

Using a lyophilised skin allograft is a method for the storage of non-viable skin allografts. Lyophilised skin can be kept at ambient temperature for years. A maximum limit for residual moisture should be defined.

17.4.8. De-epidermised dermis and acellular dermis

Using a de-epidermised dermis and acellular dermis is a method to lower the antigenicity of the skin graft. Thicker skin obtained from deceased donors is processed aseptically to remove the epidermis and dermal cells that can led to tissue rejection and graft failure.

Complete de-cellularisation of the dermal matrix requires incubation in detergents or enzymatic extraction methods as well as nuclease buffer treatment to remove residual nuclear materials [9]. Full-thickness skin obtained from living donors undergoing abdominoplasty or body-contouring procedures can be 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 [10]. The result is an intact dermal matrix that can be cryopreserved, preserved in glycerol or lyophilised. See Chapter 20 for information on the use of de-cellularised tissues as natural extracellular matrices.

17.4.9. Sterilisation of skin allografts

When tissue viability is not required or where skin tests positive for microbiological contaminants, it can be sterilised by gamma-irradiation. Research has shown that 25-kGy irradiation of deep-frozen skin in radio-protective solutions sterilises tissue without histomorphological or physical alterations compared with normal cryopreserved skin [11].

17.5. Quality control

17.5.1. Microbiological testing of skin

Microbiological testing should be done before the start of processing and on post-processed samples of skin (without antibiotic) for each anatomical area. Specimens should be obtained directly from the site that has been processed and placed in a specimen container labelled with the anatomical area (back, leg, etc.). These specimens should be sent for culturing to check for bacteria and fungi. Acceptance criteria regarding microbial load and types of contaminants in processed tissues should be defined in advance and reported in written procedures. Specimens contaminated by critical pathogens, such as *Clostridium* sp., should be discarded without corrective actions in order to remove potentially unsuitable tissue from the transplantation process.

Processing facilities should have a robust quality control/assurance programme that has a validated process to eliminate or reduce potential pathogens that may cause disease transmission. This should include evaluation of the bacteriologic bioburden (for pre-processing and in-processing samples to evaluate contamination), validated bacteriologic and/or virucidal washes and/or treatments, final product

testing for microbiological contamination using destructive testing and a final review of screening/testing by the Responsible Person (RP) of the tissue establishment before release of the tissues for transplantation.

17.5.2. 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 any national regulations.

17.6. Examples of serious adverse reactions and serious adverse events

The Notify Library 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 whereas, 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.

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Chapter 18

Cardiovascular tissue

18.1. Introduction

Cardiovascular tissues can be procured from deceased heart-beating and non-heart-beating donors and from living donors (e.g. heart valves from patient undergoing a heart transplant when the indication for their transplant procedure is not a valvular disease, saphenous veins).

Due to the greater resistance to infection of allografts compared with synthetic materials, the current most important clinical indication for adults is the replacement of infected valves (endocarditis) and the replacement of infected vascular prostheses. For valve replacement in newborns and young patients, human valves are usually transplanted to repair congenital malformations. They are the preferred option as they avoid the need for long-term anti-coagulant therapy (as required for the mechanical alternative) and do not tend to calcify as rapidly (as do the xenograft alternatives). In the case of arterial tissues, peripheral re-vascularisation and arterial patches for repair of congenital malformations are the most common indications.

The future of banking of cardiovascular tissue is to develop new procedures (e.g. decellularisation of such as valves and blood vessels) to allow recellularisation with cells from the recipient *in vitro* before

implantation or *in vivo* after the tissue implantation. Tissue engineering (i.e. combining synthetic materials with decellularised human matrices), and other advanced therapy procedures represent important technical improvements for banking of cardiovascular tissue.

The following generic chapters of this Guide (see Part A) all apply to cardiovascular 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 (Chapter 3)

d. Donor evaluation (Chapter 4)

e. Donor testing (Chapter 5)

f. Procurement (Chapter 6)

g. Processing and storage (Chapter 7)

h. Principles of microbiological testing (Chapter 8)

i. Distribution and import/export (Chapter 9)

j. Organisations responsible for human application (Chapter 10)

k. Computerised systems (Chapter 11)

l. Packaging and labelling (Chapter 12)

m. Traceability (Chapter 13)

n. Biovigilance (Chapter 14)

This chapter defines the additional specific requirements for cardiovascular tissue.

18.2. Donor evaluation

18.2.1. Contraindications specific for 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 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;

e. bacterial or fungal endocarditis.

Other conditions to be evaluated as part of the donor selection process are:

a. chronic alcoholism with myocardial dilatation;

b. pneumonia in previous days without evidence of effective treatment;

c. previous cardio-surgical interventions on the tissue to be procured.

Common practice is to procure vessels from donors aged <55 years and heart valves from donors aged <65 years. However, some centres are validating the extension of these limits based on the specific evaluation of the quality of the tissue.

18.3. Procurement

18.3.1. 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 wear sterile gloves, face shields and protective masks.

18.3.2. Procurement procedure

For heart valve procurement it is important, whenever possible, to procure the ascending aortic artery (including the supra-aortic trunks and the pulmonary artery with the complete bifurcation) together with the heart.

For vessel donors, the maximum possible length of the recovered vessel should be maintained, avoiding iatrogenic lesions during manipulation, and collateral branches should be cut 2–3 mm from the arterial wall.

18.3.3. Tissue transportation to the tissue establishment

Common practice is to place procured tissues in a crystalloid transport solution (e.g. Ringer's lactate solution, Hank's balanced salt solution) with the possible addition of nutritional/osmotic elements (e.g. albumin) or antibiotic cocktail, and packaged in at least two sterile packaging layers after procurement.

For donors of organs, valves and vessels, it is convenient to package the heart and the vascular segments in different containers.

This package should then be placed in another container that ensures a temperature of 2–10 °C and protects the recovered tissues during transport.

18.3.4. Procurement documentation

The organisation responsible for procurement must produce a procurement report to be given to the tissue establishment. In addition to the generic requirements defined in Chapter 6, this report must contain a description and identification of the recovered material (heart, arteries, veins, valves, etc.).

18.4. Processing and storage

Procured cardiovascular tissues can be processed to facilitate longer storage periods and to reduce microbial contamination.

To ensure tissue quality, it is essential that the time between cardiac arrest and cryopreservation be as short as possible. Cardiovascular tissue should be procured within 24 h after death if the body has been refrigerated in the first 6 h after death or within 15 h after death if the body has not been refrigerated. Time from procurement of the heart to dissection and disinfection should not exceed 24 h.

Processing of cardiovascular tissues includes dissection and evaluation of minimum functional requirements, incubation with antibiotics and, in some cases, anti-mycotics, cryopreservation and storage. The duration and temperature of antibiotic treatment and the composition of antibiotic cocktails should be defined by each tissue establishment, with prior evaluation of the initial tissue bioburden (i.e. before the tissue comes into contact with an antibiotic solution) and following a validation of the effectiveness of the cocktail against the most common microbes likely to contaminate the tissues.

The methods used must be in accordance with current state-of-the-art and validated procedures (see Chapter 3). Different tissue establishments apply specific preparation processes according to their own standard operating procedures (SOP) and in accordance with relevant local authorisations.

18.4.1. Decellularisation of cardiovascular tissues

Heart valves and large vessels can be decellularised employing different methods to eliminate cellular components. Decellularisation protocols employ physical methods (freezing, sonication), chemical methods (hyperosmotic solutions, ionic detergents, non-ionic detergents) and enzymatic methods (trypsin, endonucleases). The most robust and effective decellularisation protocols include a combination of the three methods described.

Quality control should guarantee the maintenance of the structure and the biomechanical properties of native valves and vessels as well to demonstrate *in vivo* function. Decellularisation protocols (especially enzymatic methods) should take into account that degraded

collagen might have repercussions for *in vivo* re-endothelialisation of decellularised tissues.

See Chapter 20 for additional information on decellularisation methods.

18.4.2. Processing facilities

In selecting an appropriate air-quality specification for processing of cardiovascular tissue, the criteria identified and explained in Chapter 7 should be considered. Table 18.1 outlines factors to be considered for processing of cardiovascular tissue.

Table 18.1. Factors influencing the specification of processing air quality for 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.
Use of antimicrobials during processing	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 as this method is not very effective compared to more robust methods that can be applied to other tissues.
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 rinsates from washings should also be sampled to make this evaluation more effective.
Risk of transfer of contaminants at transplantation	Cardiovascular tissue is vascularised and can support and transmit microbiological contaminants, bacterial and viral agents. 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.

It is vital that the processing of cardiovascular allografts take place in a microbiologically and physically controlled environment with temperature control, ventilation and air filtration, and with validated cleaning and disinfection. Taking the factors from Table 18.1 into consideration, cardiovascular tissue should be processed in optimal environments with an air quality of Grade A (European Union [EU] Good Manufacturing Practice [GMP] Guidelines with an adequate background environment. For EU countries, the background must be at least Grade D (EU GMP), but given the risks associated with the processing, testing and implantation of cardiovascular tissues, it is recommended that a Grade B background environment (EU GMP) be provided.

18.5. Cryopreservation and storage in liquid nitrogen

Cardiovascular tissues can be cryopreserved by using a controlled rate freezer and following a validated protocol. During the cryopreservation process, the parameters of the freezing cycle must be recorded, as well as any inconsistencies that might have occurred during the operation. After cryopreservation, the frozen tissues can be transferred to a temperature-monitored liquid nitrogen tank and stored either in the vapour phase of liquid nitrogen below –135 °C). Some tissue establishments use ultralow temperature electric freezers working below –135°C. Cardiovascular tissue can be stored in liquid nitrogen (vapour phase or liquid) for ≤5 years. Longer storage periods should be validated.

18.6. Cardiovascular tissue thawing

Thawing, the 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 of thawing, dilution and tissue reconstitution that was carried out, together with a comprehensive list of the materials used.

18.7. Quality control

It is recommended that the quality control tests on vascular grafts should consider the following minimum quality criteria:

a. integrity of the vascular walls;

b. minimal calcification, atheroma and fibrosis;

c. anatomical suitability: non-aneurism or non-stenosis accepted.

Quality control tests for heart valves should consider the following minimum quality criteria:

a. Functional competence. It should be noted that fenestrations within the margins of the lunulae are very often not a pathological finding. Provided the coaptation of the graft is ensured by adequate sizing, marginal fenestrations should not induce valve regurgitation either in the short or long term. Large fenestrations, particularly when they are in opposing cusps, should constitute a rejection criterion;

b. Good morphology (no fissures, congenital defects, no/minimal calcification, etc.). Only small calcifications in the distal wall of the aorta, where they are most likely not to interfere with graft functioning, can be accepted; although information on their size and location must be clearly reported to the clinical user;

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 8), 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 cryopreservation solution.

Table 18.2. Suggested list of contaminants that should result in tissue discard if detected at any stage of processing

Clostridium sp. (notably *C. perfringens* or *C. tetani)*
Enterococcus sp.
Flavobacterium meningiosepticum
Klebsiella rhinoscleromatis
Listeria monocytogenes
Neisseria gonorrhoeae
Nocardia sp.
Pseudomonas aeruginosa or *P. pseudomallei*
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Salmonella sp.
Shigella sp.
Streptococcus pyogenes (Group A)
Aspergillus sp.
Candida sp.
Mucor sp.
Penicillium sp.
Other yeasts and fungi
Mycobacteria sp.

The result of the microbiological control must be negative. If a positive microbiology result is obtained, depending on the microorganism

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 18.2 suggests micro-organisms which, 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* sp. 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.

18.8. Cardiovascular allograft distribution

Transportation of cardiovascular tissues can be carried out using dry-shipping containers (vapour phase nitrogen below –135 °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 re-stored at –80 °C, then the expiry date must be reduced to a maximum of 6 months. If dry ice is used for transportation of the vascular allograft, the tissue should not be returned to liquid or vapour phase nitrogen tanks, but must be re-stored at –80 °C and the expiry date reduced to a maximum of 6 months. Once cardiovascular tissues have been thawed, they cannot be re-frozen.

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.

18.9. Examples of serious adverse reactions and serious adverse events

The Notify Library includes some well-documented cases of adverse occurrences in 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 (Record Number 564). 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 of which resulting in delay in treatment and graft loss.

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.

Chapter 19

Musculoskeletal tissue

19.1. 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' bone-grafting material because it combines all the properties required in a bonegraft material: osteoinduction (bone morphogenetic proteins [BMP] and other growth factors), osteogenesis (osteoprogenitor cells) and osteoconduction (scaffold). However, use of autografts is limited by the amount that can be procured and, 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 deceased heart-beating and non-heart-beating donors and from living donors (e.g. in the case of a patient undergoing hip or knee prosthesis surgery), and include bones, ligaments, tendons, meniscus, cartilage and other soft tissues (e.g. fascia lata). The current indications for the implantation 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 methods include cutting or grinding into morcellised and cancellous chips, corticocancellous rings and wedges and cortical grafts such as struts and cylinders. The preparations can be frozen or freezedried, and insome cases preparations are demineralised to enhance the osteoinductive properties (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 de-cellularisation or cell therapy to improve graft incorporation in recipients;

c. improving the safety of musculoskeletal grafts.

The following generic chapters of this Guide (see Part A) 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 (Chapter 3);

d. Donor evaluation (Chapter 4);

e. Donor testing (Chapter 5);

f. Procurement (Chapter 6);

g. Processing and storage (Chapter 7);

h. Principles of microbiological testing (Chapter 8);

i. Distribution and import/export (Chapter 9);

j. Organisations responsible for human applications (Chapter 10);

k. Computerised systems (Chapter 11);

l. Packaging and labelling (Chapter 12);

m. Traceability (Chapter 13);

n. Biovigilance (Chapter 14).

This chapter defines the additional specific requirements for musculoskeletal tissue.

19.2. Donor evaluation requirements

General exclusion criteria are described in Chapter 4. Musculoskeletal tissue specific are described below.

19.2.1. Physical evaluation

The body of the deceased donor must be inspected before starting the procurement procedure to check for contraindications. The entire body should be checked for bone or joint deformities, open fractures or surgical scars in addition to the checks described in Chapter 4. The results must be recorded and taken into account during the procurement and processing phases, as well as before the final release of the tissue. Physical examination of the living donor should also be carried out.

19.2.2. Specific contraindications for musculoskeletal tissue

In addition to the general exclusion criteria described in Chapter 4, screening of donors of musculoskeletal tissue should be conducted for:

a. diffuse connective tissue;

b. metabolic bone diseases (severe osteoporosis, osteoprosis, Paget disease, etc.);

c. ingestion of cyanide or heavy metals (mercury, gold, etc.);

d. radiation exposure at the location of the tissue to be donated;

e. evidence of trauma (e.g. open fracture) at the procurement site;

f. iatrogenic, degenerative tears or lesions detected during procurement of cartilage, menisci, tendons and osteoarticular grafts.

19.2.3. Age limits for donors

Donor age limits differ for different types of musculoskeletal tissues. These limits may be revised upon performance of a validation study. Some countries have national guidelines or requirements but, in their absence, the following age limits, for male or female donors, are recommended:

a. no upper age limit is applied for donors of cancellous bone;

b. for bone, the minimum age for both sexes is 15 years;

c. for long bones, there is no upper age limit unless the bone is to be used for structural support, in which case younger donors (age 15–55 years) are preferred, due to the risk of osteoporosis (see 19.2.2.b).;

d. for osteoarticular grafts, cartilage and menisci, the age range is 15–55 years;

e. for tendons and fascia lata, the age range is 15–65 years.

19.3. Procurement

General principles of procurement are described in Chapter 6.

19.3.1. Time limits for procurement

Procurement is usually undertaken as soon as possible after the death of the donor. In the case of organ and tissue donation, procurement would be immediately after removing the heart, liver, or kidneys. It is recommended that time limit for musculoskeletal procurement is 24 h if the body has been refrigerated after death. Tissue excision shall commence within 15 h of death if the deceased donor has not been cooled or refrigerated [1, 4]. If the body has been cooled for a period of time and then not cooled for a period of time, the time period the body is not cooled must be up to 15 consecutive cumulative hours.

19.3.2. Procurement from deceased donors

The methods of tissue collection may be similar to those used by orthopaedic surgeons in the operating room or may use wider skin incisions, applying strict aseptic technique. The procurement team should work under aseptic conditions, and be scrubbed, gowned in sterile clothing and wear sterile gloves, face shields, glasses or protective masks. Musculoskeletal tissues must be procured after the donor has been washed, followed by appropriate pre-operative disinfection of skin to reduce transient and resident microbial flora. A local sterile field using disposable sterile drapes must be established before procurement to effectively reduce microbial contamination. The donor's body is prepared and draped in a similar fashion to that used during surgery. Cultures are obtained at least once before further processing of the tissues recovered.

Limiting the number of members of the procurement team can be critical to minimising the risk of contamination tissues during procurement [5]. It is important to define the functions of the different members of the team for the procurement process to avoid cross-contamination.

Staff must have the experience, education and training necessary to procure tissues, including significant anatomical knowledge to accurately obtain not only the regular tissues recovered (femur, patellar ligaments, etc.), but also specially requested materials (e.g. whole elbow).

After procurement, musculoskeletal tissues shall be packaged in a manner that minimises contamination risk. Procured tissue must be inspected and identified appropriately before packaging and labelling to avoid mix-ups.

The musculoskeletal tissues most frequently procured from deceased donors are:

a. long bones (femur, tibia, fibula, humerus, radius, rib);

b. flat bones (iliac crest, hemipelvis);

c. vertebrae;

d. tendons (Achilles, patellar, tibialis) without or with attached bone blocks (bone–tendon–bone or bone–tendon);

e. fascia (fascia lata) and other soft tissues (e.g. menisci).

19.3.3. Reconstruction of the deceased donor

Once tissues have been procured from a deceased donor body, the body must be reconstructed to maintain its original anatomical appearance.

For aesthetic reasons and with a view to a respectful reconstruction of the donor, a wooden or plastic replica bone approximating the size of the donated bone may be used to replace the procured bone. The subcutaneous tissue and skin should be sutured. The use of sutures and other materials suitable for cremation should be considered.

19.3.4. Procurement from living donors

Musculoskeletal tissues can also be procured from living donors.

19.3.4.1. *Allografts*

Patients having a hip replacement procedure can donate the femoral head that is being replaced by the prosthesis and in some cases bone removed in knee replacement is also banked. This can be frozen or further processed and provided to other patients as a bone graft.

19.3.4.2. *Autologous grafting*

In some cases, musculoskeletal tissues are procured and transplanted in the same patients, with a banking procedure in between. A common example is the banking of cranial flaps removed during neurosurgical procedures where there is brain oedema. The tissue is stored and replaced in the same patient once brain swelling has diminished.

19.4. Processing

19.4.1. Processing facilities

In selecting an appropriate air quality specification for musculoskeletal tissue processing, the criteria identified in Chapter 7 should be considered. Table 19.1 outlines the factors to be considered for processing of musculoskeletal tissue.

It is important that processing of musculoskeletal allografts take place in a bacteriological- and climate-controlled environment, including temperature and humidity control, ventilation and air filtration, with validated cleaning and disinfection. Where these grafts are not destined for terminal sterilisation, the need for an optimal processing environment is critical to the safety of the graft.

Within the European Union (EU), tissues that are exposed to the environment without a subsequent microbial inactivation process should be processed in environments with an air quality equivalent to those of Grade A (as defined in EU Good Manufacturing Practice [GMP]), with a background environment at least equivalent to Grade D (EU GMP). Many national requirements are more stringent, requiring Grade B (EU GMP) as a background, which may be more appropriate for the processing of bone and tendons that are prone to tissue contamination due to extensive manipulation and that have processing phases at 37 °C that are not followed by a terminal sterilisation step. Bone that is destined for terminal sterilisation can be processed in a Grade C environment.

Table 19.1. Factors influencing the specifications of processing air quality for 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 during the freeze-drying procedure.
Use of antimicrobials during processing	It is possible to soak musculoskeletal tissue in antibiotics just after procurement and before initial packaging or during processing, but these do not penetrate the tissue and can only inactivate surface contaminants. Blood and marrow removal, washing and similar preservation methods have been shown to be effective for reducing or eliminating contamination with micro-organisms. Bone can be terminally sterilised by gamma-irradiation, accelerated electron beams or with a series of washes and chemical treatments that together achieve an equivalent degree of sterility (see section 19.4.4).
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of complete structural fragments of bone or tendon is limited to taking small and unrepresentative analyses or to swabbing. Tests can be done by immersion of the tissue in culture medium after processing or by filtering and culturing wash solutions. For bone that is processed to small pieces or ground, representative samples can be taken for culturing. For terminally sterilised bone, sampling is not an issue as the process is validated to achieve a certain sterility assurance level.
Risk of transfer of contaminants at transplantation	Musculoskeletal tissue that has not been thoroughly marrow-depleted during processing is fully vascularised and the risk of transmission of viral and bacterial agents exists. Musculoskeletal tissue is used in open and well-vascularised surgeries, sometimes linked to replacement of a prosthesis, where a significant risk of infection exists.

19.4.2. Musculoskeletal graft processing

There are various methods of bone processing applied by individual tissue establishments. Allogeneic and autologous bone allografts from living donors can be processed in the same manner as for tissues from deceased donors. It is recommended to store musculoskeletal tissues between processing steps, at temperatures of –40 °C to –80 °C or, for short periods, to keep on dry ice.

Musculoskeletal grafts from different donors must not be pooled during processing.

Processing of bone and other musculoskeletal tissue generally involves the removal of extraneous tissues (muscle attachments and the periosteum) and blood as for other tissues. In addition, intraosseous blood and lipids can be removed using physical debridement, alcohol solutions, high-pressure water rinses, mechanical agitation and/or ultrasonic processes.

The protocols for these steps vary between tissue establishments.

19.4.2.1. *Treatment of musculoskeletal tissues*

a. Processing of cancellous and cortico-cancellous bone

Cancellous and cortico-cancellous bone grafts might be prepared from epiphyses of the distal femur and proximal tibia, proximal and distal epiphyses of the humerus and vertebral bodies, and the iliac crest. Long bones (femur, tibia humerus, radius, ulna) may be cut in the border between the diaphysis and epiphysis (metaphysis) using different types of saw (e.g. banding, oscillating). Then, the final shape of a graft can be achieved. The full length of long bones can also be stored.

b. Processing of osteochondral bone

Osteochondral bone grafts might be prepared from the distal or proximal femur, tibia, humerus, radius and ulna. The shape of the final graft can be achieved using different types of saw.

c. Processing of ligaments and tendons

Achilles and patellar tendons as well as ligaments may be cleaned mechanically.

19.4.2.2. *Washing and defatting procedures*

Washing and defatting procedures are used to remove cells from cancellous and cortical bone tissue. During mechanical and chemical cleaning, most or all of the bone marrow is removed using, e.g. chloroform/methanol (or ethanol) solution (2:1, *v/v*), hydrogen peroxide, or warm water or saline solution at 30 °C. In-between the steps of that procedure, a rinsing solution should be used. A visual examination of the bone to check for structural integrity should be documented.

19.4.2.3. *Lyophilisation*

Lyophilisation (freeze-drying) in this context involves moisture extraction from bone by freezing and prevention of ice formation. The purpose is to permit shelf storage at room temperature. At the end of freeze-drying the available water (aW) should be <5%.

19.4.2.4. *Demineralisation*

Demineralisation comprises reducing the calcium content of bone grafts to expose the BMP that provide the osteoinductive capability of the graft, thereby improving its incorporation in the recipient. Bone is ground down (standard diameter [in microns]: 80–300, 300–425, 425–600, 600–1000) using a freezer mill. Then it is demineralised in acidic solution (e.g. 0.5 or 0.6 M HCl) for 90 min. Then, bone is defatted and alternatively lyophilised or frozen. Calcium content (usually <10%) should be calculated (e.g. percentage calcium in the bone matrix by dry weight).

19.4.3. Impact of processing on musculoskeletal allografts

Various methods of processing and shaping are used in the preparation of tissue allografts. Grafts for skeletal reconstruction (which are used in procedures requiring structural support) must be processed in a manner that does not alter their inherent mechanical properties

significantly. The preparation process of allograft material can be fresh-frozen, freeze-dried, or demineralised.

Freezing an allograft has little impact on the mechanical properties of the tissue, and will diminish its immunogenicity. In contrast to fresh-frozen allografts, mechanical strength in freeze-dried allografts is reduced significantly [3]. Freezing affects the viability of articular cartilage unless some form of cryopreservation is employed. Freeze-drying further diminishes the immunogenicity of the graft. It does, however, have some negative effects on the mechanical strength of the grafts, especially if rehydration is not done appropriately in the operating room. Freezing or freeze-drying do not appear to affect the healing properties of bone.

Gamma radiation is effective in killing bacteria, fungi, spores, and, to more variable degree, viruses. Depending on the dose, however, gamma radiation can weaken the graft. Doses <15 kGy do not seem to adversely affect tissue strength [4]. Gamma irradiation and excessive heat (>60°C) is known to damage bone proteins. The damaging effects of gamma irradiation are greatly increased for freeze-dried tissue.

The adverse effects of irradiation can be ameliorated by reducing the temperature and inclusion of radioprotectant chemicals.

19.4.4. Sterilisation or decontamination of musculoskeletal tissues and cells

Tissue can be processed aseptically or be sterilised subsequently.

Sterilisation refers to any process that eliminates/inactivates transmissible infectious agents (pathogens) containing nucleic acids (e.g. vegetative and spore forms of bacteria and fungi, parasites, viruses) [2]. For sterilisation and decontamination, a wide range of procedures can be used, including combinations of washing with or without pressurisation, centrifugation with various chemicals (e.g. alcohol, detergents) and combining antibiotics with low-dose radiation. Regardless of the method used, the objective is to remove all infectious elements with minimal impact on the performance of the allograft [4]. Sterilisation procedures should ensure that no viable organisms are present in the

sample after sterilisation. The term Sterility Assurance Level (SAL) 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 of one unit of product being contaminated with a single organism after a sterilisation process, and grafts are considered sterile.

After the grafts have been processed and cleansed, they are packaged and stored. The designated shelf-life is dependent upon the packaging and storage methods used (deep frozen, freeze-dried, fresh).

Grafts are stored at a tissue bank during the quarantine period until the required serologic and bacteriologic test results and autopsy reports (if required) are received. The tissue establishment must confirm donor eligibility before releasing the graft.

19.4.4.1. *Sterilisation*

a. Radiation sterilisation

Both gamma rays and accelerated electron beams can be used for sterilisation processes. No specific dose can be recommended as this depends on multiple factors associated with the individual process. However, it should be noted that higher doses can reduce the biomechanical strength of the bone. Doses used for gamma ray sterilisation range from 17 kGy to 35 kGy and are established after calculation of the initial bioburden of the tissue. The irradiation process must always be documented.

b. Sterilisation using ethylene oxide

Although an effective sterilant, ethylene oxide is no longer recommended for tissue processing due to the risks of residuals that might be mutagenic.

19.4.4.2. *Decontamination*

a. Chemical decontamination

There are many chemicals that can be used as decontaminants or that have an inactivating effect on specific pathogens (e.g. peracetic acid, iodophors, ethanol, etc.). The effectiveness of these agents on certain types of tissue must be validated. It is important that the chemicals used be mentioned in the documentation that accompanies the grafts, particularly if it is possible that traces of these products or their by-products remain in the tissue.

b. Antibiotic decontamination

Antibiotics may be used to decontaminate musculoskeletal tissue. The effectiveness of each 'antibiotic cocktail' should be validated and documented. The use of an antibiotic decontamination procedure might be the only method of microbial inactivation possible for grafts where cell viability is required.

19.5. Packaging and labelling

Generic requirements are detailed in Chapter 12.

Procured musculoskeletal tissues are to be packaged in a way that minimises contamination risk. It is recommended that musculoskeletal tissues be at least double-packed in airtight packages or in sterile drapes as well as sterile containers. Each procured tissue is to be packed separately and labelled immediately after recovery.

19.6. Transport and storage

19.6.1. Transport of musculoskeletal tissues

Procured musculoskeletal tissue should be transferred to a tissue bank as soon as possible. Transport temperatures correspond principally to the storage temperatures and are specified below.

In general, musculoskeletal tissues should be cooled as quickly as possible and transported at hypothermic conditions. It is recommended to use special climate controlled containers for prolonged transport. Cartilage for chondrocyte culture must not be frozen. Time for transportation should not exceed 24 h and during this time temperature can be 5–15 °C.

Transport conditions should be also followed and monitored during transport from the tissue bank to the hospital.

19.6.2. Storage of musculoskeletal tissues

In general, musculoskeletal tissues should be stored at hypothermic conditions and at temperatures appropriate to maintain their characteristics and biological functions suitable for their intended use. Each type of storage condition should specify the maximum shelf-life for the tissue, as well as exclusions for specific graft types. Storage temperatures according to validated protocols should be –20 °C to –80 °C (on dry ice, in freezers or deep-freezers). It is also acceptable to store musculoskeletal tissues in containers of liquid nitrogen between –75 °C and –196 °C [6].

Bone is commonly preserved by freezing and storage at –80 °C, or by freeze-drying. Tendons and fascia do not require living cells either, but the collagen matrix must retain adequate properties. These tissues are also commonly stored at –80°C. Cartilage for chondrocyte culture should be stored at 4–8 °C in a refrigerator.

Maximum storage periods for banks should be established by tissue establishments using validation studies.

An important limitation is the integrity of the packaging material to ensure microbiological safety and to prevent rehydration during storage. The packaging material must also be validated for storage in hypothermic conditions.

19.6.2.1. *Preservation and storage in freezers*

Preservation and storage of musculoskeletal tissues (including cancellous, cortico-cancellous and cortical bone, ligaments and tendons) by freezing is a common method applied. There is limited scientific evidence to justify particular temperature limits, but in general it accepted that:

a. temperatures from –20 °C to –30 °C allow storage for ≤6 months.

b. temperatures from –40 °C to –80 °C (deep freezers) allow tissue storage for ≤5 years.

19.6.2.2. *Cryopreservation*

Cryopreservation is a process whereby tissues are preserved by cooling to temperatures below –135 °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 sulfoxide [DMSO]) are added to the medium to prevent the ice crystal formation that destroys cells. The storage time under these conditions should not exceed 5 years.

19.6.2.3. *Preservation and storage at ambient temperature*

All kinds of lyophilised musculoskeletal allografts can be stored at ambient temperature ≤5 years if the packaging has been validated to maintain sterility and integrity for that period.

For details see Chapters 9 and 10.

19.7. Quality control

Quality control tests on musculoskeletal grafts should take at least the following minimum quality criteria into account:

a. morphology and integrity of the musculoskeletal grafts;

b. shape and size of the graft;

c. residual moisture in lyophilised grafts (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.

During procurement or before processing, microbiological samples should be collected to establish the initial contamination levels of tissues to assist in making a decision during quarantine regarding the release of procured material for further processing. These microbio-

logical tests are also important as controls during the procurement procedure.

Samples for microbiological analysis should also be collected before packaging of the final product. Possible sampling techniques for microbiological testing include:

g. swabs;

h. destructive methods (e.g. biopsy or sacrificing a proportion of ground tissue);

i. collection of the last portion of the fluid used for washing of the tissue graft for subsequent analysis, usually following filtration.

See Chapter 8 for more detailed guidance on the principles of microbiological testing and Table 6.1 for risk assessment of each sampling method.

19.8. Examples of serious adverse reactions and serious adverse events

The Notify Library includes many well-documented cases of adverse occurrences in the field of musculoskeletal tissue transplantation. Examples include:

a. 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 transplantation.
- 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 disease and rheuma-

toid arthritis respectively, were all diagnosed during histological examination of the femoral head and resulted in discarding of allografts.

b. 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 the donor and recipient.
- 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).

c. Meniscus

- Record Numbers 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.notify-library.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by Record Number.

19.9. References

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Chapter 20

General considerations for cell-based therapies

20.1. Introduction

In recent years, more and more tissue establishments have been expanding their activities, providing starting materials or engaging in the production of complex products incorporating human tissues and cells which may fall under different legal frameworks, such as transplantation, medicinal products or medical devices. This chapter includes technical considerations to professionals from tissue establishments who may be interested in developing such activities without providing any guidance on how these products are or should be regulated. Irrespective of the content of this document, any operator active in the field should carefully consider the legal requirements that apply to the activities he/she is undertaking. It is advisable that, before starting any activities, there is appropriate consultation with the relevant authorities to understand the regulatory environment and seek any license/authorisation that may be required.

Cells of human origin represent a special category of substances used in human application, some of which are already well established in medical practice, such as haematopoietic stem cells. The existence of

stem cells and their capacity to reconstitute bone marrow has allowed transplantation into patients with a wide range of life-threatening conditions for many decades. More recently, the ability to generate new tissue-specialised cells during adulthood has created great possibilities for the development of cell therapy and regenerative medicine. Most of these innovative therapies are classified in the European Union (EU) as advanced therapy medicinal products (ATMP). Specifically, it is noted that the use of cells that have been subject to substantial manipulation (such as expansion), as well as the use of cells for a different essential function or functions in the recipient as in the donor (non-homologous use), are regulated as medicinal products in the EU. This means that their processing, storage, distribution and use in patients must respect the requirements of Regulation 1394/2007 on Advanced Therapy Medicinal Products ('ATMP Regulation') as well as all other relevant provisions of the EU medicines rules. For clinical trials with ATMP, the EU and national legislation for clinical trials needs to be followed. Likewise, it is noted that the term 'cells' or 'cell therapy' may be used in this guide to refer to situations that are regulated as ATMP in the EU. This Guidance is not intended to affect the scope of the EU rules on medicines and any operator that intends to process, store, distribute or use cells in humans should first seek advice from national authorities on appropriate applicable legal framework. Additional information on the EU legal framework covering medicinal products incorporating tissues, cells and/or genes is presented in section 20.2 of this chapter.

The procurement and application of cells for a specific therapy can be autologous (from the same patient) or allogeneic (from a donor). Several different cells have been applied in humans, including haematopoietic stem cells (see Chapter 21), which are the most commonly used progenitor cells. Development of other therapies for the treatment of many diseases based on mature or immature cells of haematopoietic lineage or not is also rapidly increasing (see Chapter 22).

The following generic chapters of this Guide (see Part A) all apply to cells for human application 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. Processing and storage (Chapter 7);

h. Principles of microbiological testing (Chapter 8);

i. Distribution and import/export (Chapter 9);

j. Organisations responsible for human application (Chapter 10);

k. Computerised systems (Chapter 11);

l. Packaging and labelling (Chapter 12);

m. Traceability (Chapter 13).

In the EU, these chapters are only applicable for starting materials (tissues and cells) used for the manufacturing of ATMP.

20.2. EU legal framework

20.2.1. Advanced Therapy Medicinal Products Regulation

To provide for a common framework for the marketing of so-called ATMP, Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products (hereafter, 'ATMP Regulation') was adopted in 2007.

The Regulation applies to medicinal products (as defined by the Directive 2001/83/EC) which are one of the following:

a. a gene therapy medicinal product;[1]

1 A gene therapy medicinal product is a biological medicinal product that contains an active substance which contains or consists of RNA used in humans with a view to regulating, repairing, replacing, adding or deleting a genetic

b. a somatic cell therapy medicinal product;[2]

c. a tissue engineered product;[3]

The cornerstone of the 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 quality, efficacy and safety profile, it is demonstrated that the benefits outweigh the risks. The application for a marketing authorisation must be submitted to the European Medicines Agency (EMA) and the final decision is taken by the European Commission. This procedure ensures that these products are assessed by a specialised body (the Committee for Advanced Therapies [CAT]) and that the marketing authorisation is valid in all the EU member states.

Where the ATMP contains human cells or tissues, Directive 2004/23/EC applies to the donation, procurement and testing of the tissues and cells.

The ATMP Regulation gives member states the power to authorise the use of custom-made ATMP prepared on non-routine basis in the absence of a centralised marketing authorisation, provided that the product is used for individual patients in a hospital and under the professional responsibility of a medical practitioner. 'Hospital exemp-

sequence, provided that its therapeutic, prophylactic or diagnostic effects relates directly to the RNA sequence it contains or to the product of genetic expression of this sequence.

2 A somatic cell therapy medicinal product is a biological medicinal product that:
- contains or consists of tissues or cells that have been subject to substantial manipulation, or of tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor, and
- is presented as having properties for, or it 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.

3 A tissue engineered product is a product that:
- contains or consists of tissues or cells that have been subject to substantial manipulation, or of tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor, and
- is presented as having properties for, or it is used in humans with a view to regenerating, repairing or replacing a human tissue.

tion' requires the application of national requirements on quality, traceability, and pharmacovigilance equivalent to those required for authorised medicinal products.

Developers of products based on genes, tissues or cells can apply to the EMA for an opinion whether a specific product is an ATMP. The procedure is intended as an incentive for developers that can ascertain at an early stage of development if their product must comply with the requirements that apply to ATMP. The classification procedure is optional.

CAT is the committee at the EMA responsible for assessing the quality, safety and efficacy of ATMP that follow the centralised procedure for marketing authorisation and following scientific developments in the field. It is a multidisciplinary committee, gathering together some of the best available experts in Europe. It is recommended to contact the CAT when developing a new ATMP because one of the missions of the CAT is to assist in the preparation of any documents related to the fulfilment of the objectives of Regulation 1394/2007.

It is important to stress that, in the EU, ATMP are medicinal products. It follows that the overall regulatory framework governing medicines (including, but not limited to, provisions governing manufacture, distribution, packaging and labelling, pharmacovigilance, and advertising of medicines) apply to ATMP. Furthermore, use of ATMP in an investigational setting is also subject to the EU rules on clinical trials.

Additional information about the EU regulatory framework for ATMP can be found at the EMA [1] and EC Internet websites [2].

20.2.2. National Competent Authorities

In EU member states, human cells for human application can be subject to different regulatory frameworks (depending on the intended use, mode of action and degree of manipulation). On the one hand, cells that are not substantially manipulated (e.g. not subject to culturing) and are used for the same essential function in the recipient as in the donor are regulated under the tissues and cells Directives. On

the other hand, if the cells are intended for a non-homologous use or if they are substantially manipulated they are considered ATMP and are regulated as medicines. Advice on the classification of a specific cell therapy can be sought from the CAT [3]. In some countries, the regulatory body is the same for all cell therapy products whereas, in others, those classified as ATMP are regulated by a different body/agency than those classified as cells for transplantation.

The ethical and legal position on the use of human stem cells or progenitor cells, as well as the regulatory oversight, differs in countries around the world. In the EU, each member state is able to make decisions on the use of progenitor stem cells for basic research. However, they must be compliant with the requirements stated in the ATMP Regulation to be used for treating patients.

Some countries have national legislation concerning paediatric donors that should be taken into account when cells from children are considered.

20.2.3. Ethics Committees

An important part is played by the local/regional/national Independent Ethics Committees (IEC) designated to approve and review biomedical and behavioural research involving humans, including the scientific rationale for the clinical application. For the latter, IEC should consider the irreversible nature of some gene/cell therapies and address the acceptability of exposing a donor to a research protocol for the benefit of the recipient, in particular where the donor is a relative of the subject, specially a parent or a sibling, to be included in the trial. IEC should also check appropriate traceability and guarantees regarding subject data protection and confidentiality. Written informed consent for receiving a cellular therapy is considered a pre-requisite as in any clinical trial.

The International Society for Stem Cell Research (ISSCR) guidelines recommend that special emphasis be placed on the risk of stem cell-based clinical research during the informed consent process. This risks include sensitivities surrounding the source of cellular products,

tumour formation, immunological reactions, unexpected behaviour of the cells, and unknown long-term health effects [4].

20.3. Processing cells for human application

Cells for human application comprise a separate therapeutic technology platform to that of the current three pillars of healthcare: chemical drugs, biologics and medical devices. They certainly are innovative strategies, but cell therapy is not new. Cell therapy has its origins rooted in blood transfusion, bone marrow and organ transplantation, tissue banking and *in vitro* fertilisation.

The isolation and characterisation of stem cells from almost all body tissues, including the nervous system, has presented new opportunities for cell therapy. Stem cells have unique characteristics that can be used to support different approaches to the cure of specific diseases. However, before the development of a new cell-based therapy it is necessary to understand the physiological properties of each stem cell or progenitor cell.

20.3.1. Special safety considerations when 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 the embryo culture, genetic manipulation, nuclear transfer, derivation, expansion of cells, addition of cryopreservatives and all steps involved in the culture of cells must be undertaken in an environment that is fully compliant with good manufacturing practices (GMP).

Culture conditions are critical during expansion and differentiation of cells in culture. 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 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 8).

The validation of the preparation process with respect to maintaining genetic stability and the relevant biological properties, as well as avoidance of malignant transformation, should be carried out.

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 xenomaterial should be avoided as much as possible by using human derived factors, for example obtaining serum from the intended recipient. However, if animal materials cannot be avoided, specification and verification of the source and method of preparation of the material is required. Culture media and other reagents derived from animals must be evaluated for the risk of contamination with micro-organisms, particularly viruses and agents of transmissible human pathologies such as transmissible spongiform encephalopathies (TSE). 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, culture media, reagents 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. The requirements for sourcing/donation, procurement and testing are set out in Annex 2 of the GMP Guidelines and in the guideline on xenogenic cell-based medicinal products. For further guidance on cell culture, refer to the report on the second European Centre for the Validation of Alternative Methods (ECVAM) Task Force on Good Cell Culture Practice [5].

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/volume reduction of the collected cells, washing or clarification of the collected cells, formu-

lation of the cells into an appropriate media for biopreservation and filling the formulated cells into their final container 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.

In the EU, this activity is governed by the medicines rules, which provide for specific requirements that must be respected when the activity is undertaken in the EU or if the cultured cells are intended to be used in the EU.

20.3.2. Microbiological testing when culturing cells

In this section we discuss practical microbiological testing when culturing cells in a GMP facility. For the microbiological monitoring of the processing facility please refer to Chapter 7 and for technical microbiological testing methods see Chapter 8.

Culturing cells in a GMP facility includes a broad range of procedures that differ in many aspects such as source material, finished product, culture reagents, time in culture, expansion, differentiation, quality control analysis and storage procedures between others. All these differences make it difficult to establish a general rule for microbiological testing. Therefore, for each procedure it would be necessary to establish a Quality Risk Management (QRM) to determine the quality control strategy to follow through the whole process to identify critical steps and 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

European Pharmacopoeia (Ph. Eur.). If other methods are used, they must always be validated in advance.

When the starting material, including the cells themselves, cannot be stored in quarantine until the results of the microbiological analysis are obtained (due to instability reasons), the processing steps can start without the results being available. However, it would be necessary to study the potential risks of using this 'non-tested material' and document it following the principles described in the QRM.

At the end of the manufacturing process, always undertaken in aseptic conditions in a GMP facility, the finished product should be analysed for its microbiological quality before it can be released. Absence of bacteria, fungi and mycoplasma should be demonstrated. However, in specific cases when this approach is not feasible, the release of the finished product before getting the microbiological analysis results may be justified. Therefore, it will be necessary to implement and document an adequate processing which may provide sufficient assurance of the microbiological quality of the product when released. This will include in-process microbiological tests that have been established based on a risk analysis. It usually includes the analysis of the sterility of the culture media as well as of samples from the intermediate product at critical steps. It is recommended to use tests as described in Ph. Eur. 2.6.1 and 2.6.27. Nevertheless, these methods are time consuming and results are available after 7-14 days depending on the method used. Alternative methods have been developed in recent years and some of these methods have shown potential for real time or near-real time results (see Chapter 8). Absence of Mycoplasma should also be analysed at given critical steps (Ph. Eur. 2.6.7.) and Endotoxin determination may also be recommended (Ph. Eur. 2.6.14.).

In principle, the microbiological analysis should be done either in the cell source material, intermediate-cell products or finished product. However, when the number of cells is scarce it may be acceptable to perform the analysis with samples of washing media, supernatants or culture media of the cells. If any microbiological contamination is detected it is mandatory to identify the strains and to investigate the

source of the contamination. In this case the product should be discarded for clinical application, however, in some cases the risk–benefit ratio should be considered.

When cells are allogeneic it is recommended to include the analysis of adventitious viruses both in cell source material as well as in finished product in order to avoid patient cross-contamination (see Ph. Eur. 5.1.7 for viral safety). Adventitious viruses can be analysed by different methodologies but PCR assays are the most common. In order to establish the virus that should be tested, it will be also necessary to do a risk analysis. Some examples of adventitious virus that can be included are: adeno-associated virus (AAV), adenovirus, bunyavirus, cytomegalovirus (CMV), Epstein–Barr virus (EBV), flavivirus, hepatitis A virus (HAV), hepatitis B virus (HBC), hepatitis C virus (HCV), human herpes virus 1 (HHV1), HHV6, HHV7, HHV8, human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2), human papilloma virus (HPV), human rotavirus (HRV), human T-cell leukaemia virus type-1 (HTLV-1), HTLV-2, influenza, measles, Norwalk virus, parvovirus B19 (Parvo-B19), rubella, and enterovirus (polio).

In the EU, this activity is governed by the medicines rules, which provide for specific requirements that must be respected when the activity is undertaken in the EU or if the cultured cells are intended to be used in the EU.

20.3.3. Considerations for quality control

In most of the cases, the short period of time between cell culture, release from the laboratory and grafting into the patients presents challenges for performing quality control tests on the final product before release. However, quality control testing and inspection during the production process and before release should be undertaken. It is important to demonstrate that the cell-based product meets specifications before release. For this reason, quality controls should include not only cell viability and genomic studies, but also cell identity and testing of biological activity and other microbiological controls. When possible, fluorescence activated cell sorting (FACS) to analyse the

presence or absence of specific cell-surface antigens (CD) is the best method to quantify the purity of the culture. For example, haematopoietic stem cells should be positive for CD34 whereas mesenchymal stem cells are negative for the antigen CD45 but positive for CD105, CD73 and CD90 (see Chapter 22 for specific information on cell surface antigens of several somatic stem cells).

The selection of appropriate markers is fundamental for the standardisation of isolation conditions and to control cell populations, heterogeneity and yield. However, in many cases there are not known specific surface antigens for stem cells, which difficult their purification.

Control of genomic stability is also critical before releasing the cells for transplants in humans. Telomerase activity, proliferative capacity and senescence are also quality controls of relevance for human pluripotent stem cells.

In the EU, quality control of cells that fall under the definition of ATMP is subject to specific requirements, which must be followed when the activity is undertaken in the EU or if the cells are intended to be used in the EU. In case of doubt whether the cells are regulated under ATMP, a scientific recommendation from the CAT can be requested.

20.3.4. Master cell banks and working cell banks

Some cells, including stem cells, are used to produce master cell banks which are then banked for future use as starting materials to manufacture cell therapy products. A cell line is established from a single clone and this cell line is used to make-up the master cell bank. This master cell bank must be characterised and extensively tested for contaminants such as bacteria, fungi, and mycoplasmas. In addition, sterility and endotoxin testing, as well as polymerase chain reaction (PCR) testing for viruses, may be required.

Cell lines used for patient treatment are placed in cryovials and cryopreservative products are added before the cells are frozen and stored in the vapour phase of liquid nitrogen. Several cryopreservatives can be used to avoid cell death during freezing/thawing procedure and to

increase cell attachment and survival after thawing. This is of special importance during freezing/thawing methods of human pluripotent stem cells [6]. The control of freezing ramp is also essential for maintaining cell viability after freezing/thawing procedures. The temperature inside the liquid nitrogen tank should be continuously monitored in order to ensure that the cells are stored under stable conditions.

It is recommended that master cell banks are stored in two or more widely separated areas within a production facility as well as at a distant site in order to avoid loss of the cell line. Dual-site storage of all cells is accomplished with on-site storage and off-site storage.

Quality controls should be the same that the described above for cell release (section 20.3.3) and carried out before cryopreservation and after thawing to ensure that cell proliferation and viability, genome stability and purity of the culture have not been affected in the freezing process.

The working cell bank is a pool of expanded well-characterised cells derived from the master cell bank. The working cell bank is prepared from a single homogeneously mixed pool of cells. One or more of the working cell bank containers is used for each cell expansion. The characterisation and quality controls carried out for the working cell bank after cell thawing must be exactly the same done for the master cell bank and the specifications should be maintained.

In the EU, this activity is governed by the medicines rules, which provide for specific requirements that must be respected when the activity is undertaken in the EU or if the cultured cells are intended to be used in the EU.

20.3.5. Safety considerations when applying cells to patients

Many of the early clinical successes using intravenous transplantation of some stem cells have been seen in the treatment of systemic diseases like graft *versus* host disease and sepsis. However, it is becoming more accepted that diseases involving peripheral tissues, such as inflammatory bowel disease, may be better treated with methods that increase the local concentration of cells. Direct injection or placement of cells

into a site for tissue repairing 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 not only resulting from culturing or *ex vivo* activation, but also as consequence of their transplantation. When cells are transplanted the environment will change considerably and these changes can modify the morphological and functional characteristics of the cells. Post-grafting follow-up of each patient is critical in autologous and allogeneic application.

At the clinical site, it is very important to educate the professional that will administer the cells to the patient. Cells are not classical drugs and need specific requirements when manipulated. It is important that administering professionals have some basic knowledge about cells. Some clinical trials failed because of a lack of training of the professionals that manipulate the cells before and during transplantation. 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.

Evaluation of tumorigenicity should also be integrated when somatic stem cells are implanted into the patient.

In the EU, use of cells falling under the definition of ATMP is governed by specific requirements and procedures, including prior authorisation by the competent authority. Evaluation of risks and benefits and the determination of the necessary data to demonstrate efficacy and safety are to be done in accordance with the EU rules on medicines if the use takes place within the EU territory.

20.4. Genetic modifications of cells

In many therapeutic strategies cells are genetically modified to induce cell differentiation, selection or to act as carriers for gene delivery. Inserting recombinant genes into cells allows the design of new strategies to treat a large variety of diseases, including genetic disorders, cancer or long-term diseases.

The term gene therapy derives from the idea that nucleic acids can be used to supplement or alter genes within an individual cell as a therapy to treat disease. A recombinant gene is a stretch of DNA that is created in the laboratory, bringing together DNA from different sources.

'*Ex vivo* gene therapy' refers to cellular modifications to introduce nucleic acids that lead to a therapeutic effect. Gene therapy products also include those nucleic acids that have other purposes, such as silencing the expression of pathological proteins. Thus these gene-based therapeutic products should include an active ingredient that contains a recombinant nucleic acid, or is constituted by it, used in humans, or administered to them, to regulate, repair, replace, add or remove a gene sequence.

Detailed guidance on this topic is not provided in the present edition of this Guide.

20.5. Natural scaffolds for clinical use

Extracellular matrices (ECM), also known as 'scaffolds', may be used directly for tissue implantation. To use scaffolds obtained from natural sources, such as human tissue, removal of all viable cells from the tissue is necessary. The resulting cellular neutralised parenchyma gives several advantages to effectively restoring unhealthy, missing or damaged membrane. For example, donated tissue may contribute to immunological rejection, but applying this technique may render the transplant successful. Once tissues are recellularised, they can be applied to patients for some therapies, for example: heart valves, large vessels or dermal matrix that can be decellularised employing different techniques (see Chapters 17 and 18).

For some development of some therapies, cells may be combined with ECM to improve or replace biological tissues. These techniques are generically defined as tissue engineering and in the EU are regulated as medicines under the ATMP Regulation.

In the EU, tissue engineered products and combined ATMP (including the manufacturing, storage, distribution, labelling, advertising, trace-

ability and use thereof) must comply with the relevant requirements provided under the EU medicines rules.

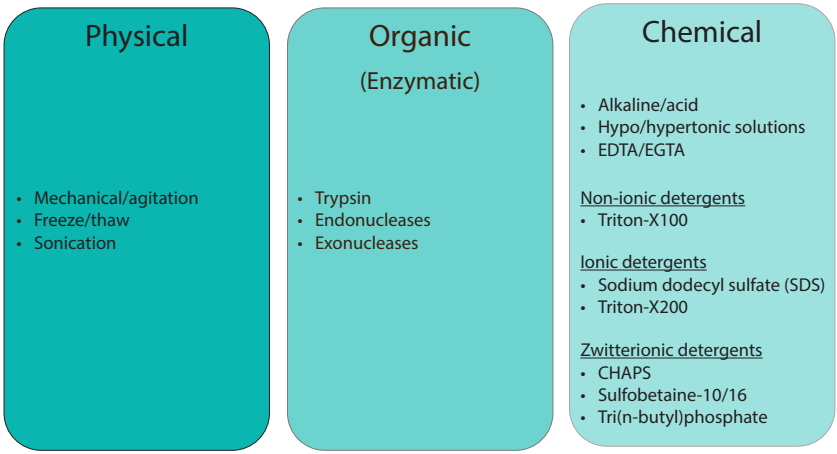
20.5.1. Donor selection and procurement

Organs and tissues for decellularisation process are mainly obtained from deceased donors. Donor evaluation and testing criteria as described in Chapters 4 and 5 apply.

20.5.2. Decellularisation agents

A clinical-grade ECM should preserve the intrinsic properties of the ECM, while removing cellular debris and donor antigens (to minimise immunological rejection) and achieving complete sterilisation and toxin elimination. However, it must be acknowledged that any decellularisation process, in order to permit the efficient removal of cells, carries a certain degree of ECM denaturation.

Figure 20.1. Decellularisation methods: physical, enzymatic and chemical



The specific decellularisation technique must be chosen depending of the final use of the ECM. ECM properties vary between tissue and

organs and the main properties to safeguard have to be clearly identified in order to choose the correct decellularisation protocol. There are three different methods to decellularise organs or tissues: physical, biological (enzymatic) and chemical (see Figure 20.1). Each of these mechanisms have different modes of action and effects on the ECM (Table 20.1). To ensure effectiveness, using a combination of methods is recommended.

Table 20.1. 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

The effectiveness of tissue and organ decellularisation depends on intrinsic tissue properties, such as the specific cell density, thickness and compaction, lipid content as well as on the selected decellularisation agents (Table 20.2.).

20.5.3. Considerations for choosing the appropriate decellularisation agent

The different decellularisation processes have different effects on the ECM properties (Table 20.2), which can be critical for the functionality of the ECM. Thus, some considerations should be taken into account when choosing the decellularisation method.

Table 20.2. Mode of action and the effects of different decellularisation methods

Method	Mode of action	Effects on ECM
Physical		
Snap freezing	Intracellular ice crystals disrupt cell membrane	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	Can lyse cells. Facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Chemical		
Alkaline/acid	Solubilises cytoplasmic components of cells; disrupt nucleic acids	Removes glycosaminoglycans (GAG)
Hypo/hypertonic solutions	Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively removes cellular remnants
EDTA/EGTA	Chelating agents that bind divalent metallic ions, thereby disrupting cell adhesion to ECM	No isolated exposure, 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

Method	Mode of action	Effects on ECM
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
Triton X-200		Yield 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		Yielded 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 was minimal
Enzymatic		
Trypsin	Cleaves peptide bonds on the C-side of Arg and Lys	Prolonged exposure can disrupt ECM structure, removes laminin, fibronectin, elastin and 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

Method	Mode of action	Effects on ECM
Exonucleases	Catalyse the hydrolysis of the terminal bonds of ribonucleotide and deoxyribonucleotide chains	

If the three-dimensional structure and its stiffness are important, collagens have to be preserved. In this case, ionic detergents would be the optimal choice, and enzymatic or alkaline-acid methods should be avoided. This consideration is valid if the preservation of any protein is important. It is worth to consider that the ECM should always be able to resist the stress inherent to the transplantation procedure.

Osmotic buffers are a milder method to obtain decellularised ECM but they are slower and cannot penetrate into thicker compact organs, such as a heart. For dense tissues or full organs, detergents can help buffers penetrate, but they will affect the proteic ultra-structure due to disruption of protein–protein interactions. Furthermore, any residual detergents can have cytotoxic effects.

Alcohols and other solvents can very efficiently remove lipids from tissues, but they can crosslink proteins and modify the ECM ultrastructure.

Enzymatic treatments guarantee the specific removal of certain proteins, but they may cause the unspecific digestion of unwanted targets and may not be sufficient for a complete decellularisation of the whole tissue. Hence they must be used in combination with other methods.

Physical methods (such as temperature, force, pressure and electricity) can be applied but have limited efficacy and should be carefully evaluated to assess any possible damage to the ECM.

Any specific combination of mild physical, biological and chemical methods, along with the type of administration, should be tested to obtain the best results for each tissue or organ.

20.5.4. Quality controls

The quality control of tissues after any decellularisation process must be the same as those applied to tissues for transplantation. However, tissue that is not appropriate for direct transplantation may be used for decellularisation purposes. It is important to determine the time available to decellularise the tissue if a pre-decellularised stored tissue or a fresh '*ad personam*' tissue will be used.

All requirements for tissue establishment apply, from the donation to the end of the decellularisation process (see Chapter 3).

20.5.5. Sterilisation of natural scaffolds

To avoid patient infection or infection of the cell culture when the scaffold is recellularised or decellularised it must be adequately sterilised. Different techniques can be applied. Classical sterilisation methods, such as ethylene oxide exposure, gamma irradiation, and electron beam irradiation, are known to alter ECM ultrastructure and mechanical properties, which are critical for ECM functionality. Ethylene oxide treatment can cause undesirable host immune responses that impair proper function of the biologic scaffold after implantation. Therefore, sterilisation by ethylene oxide is only acceptable when there is no other suitable alternative (*Ph. Eur.* 5.1.1.).

Natural ECM can be sterilised by simple treatments with acids or solvents, but such methods may not provide sufficient penetration depending on the size of the organ or tissue, and may damage key ECM components. An important issue is if the decellularisation and sterilisation processes can be coupled in one process to ensure a clinically safe ECM for the recipient without affecting its ultrastructure. At the moment, peracetic acid has been shown to be the best solution to minimise bacterial, fungal and spore content. Tributyl phosphate organic solvent has shown viricidal properties.

Supercritical carbon dioxide is under investigation as an alternative method for the sterilisation of natural ECM. 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, are 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 8 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 to eliminate endotoxins and 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. However, careful evaluation to avoid affecting the mechanical or biological properties of the ECM should be made.

20.5.6. Packaging and distribution

Decellularised tissues can be packaged and distributed for direct transplant. However, it may also be recellularised with specific cells depending on the purpose. If the decellularisation and recellularisation processes are done in the same tissue establishment, packaging of the ECM may not be needed. However, when the two processes are done in different tissue establishments, packaging is required after decellularisation.

When decellularised tissue is re-cellularised, packaging and distribution must ensure appropriate preservation of the scaffold and viability and function of the combined cells.

20.6. References

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Chapter 21

Haematopoietic stem cells

21.1. Introduction

Haematopoietic stem cell (HSC) 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 half a century ago, the procedure rapidly established itself as a life-saving treatment for a variety of malignant diseases. HSC transplantation also has a role when the haematopoietic tissue is functionally damaged by congenital or acquired disorders such as severe congenital immune deficiencies or severe acquired aplastic anaemia. More recently, the use of autologous HSC transplantation in combination with immunosuppressive 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 HSC transplantation exerts its beneficial effects through the recognition of residual tumour cells in the recipient by donor-derived immune effectors. Thus, allogeneic HSC transplantation represents one of the rare forms of clinically useful, immune cellular therapies. 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 the liver.

The field has developed tremendously in the past half-century in developed countries, and now many emerging countries are establishing allogeneic and autologous HSC transplantation programmes. The field has integrated medicinal and technical innovations, including the use of new immuno-suppressive agents, the use of different sources of HSC, such as bone marrow, mobilised peripheral blood and cord blood, the procurement of cells from unrelated donors, and much improved supportive care for recipients. Several other biotechnological advances, including stem cell selection, immune effector activation and stem cell expansion have become available. However, these advances have only entered clinical practice to a limited extent, and procurement of HSC transplantation remains relatively unchanged. Hospitals that care for recipients often obtain autologous or allogeneic HSC (which are regulated differently from other medicinal products) from hospital-based or blood establishment-based collection and processing facilities that are located in their immediate vicinity. Each of these facilities works on a typically small to medium scale. Given the high rate of international exchange of donated HSC material, harmonisation of the practices in this field would be of great benefit.

The cells discussed in this chapter are regulated in the European Union (EU) under the tissues and cells Directives (2004/23/EC and its associated technical Directives). It should be noted, however, that if these cells are subjected to substantial manipulation (such as expansion), or are used in the recipient for an essential function that is different to the original function in the donor, in the EU they are then regulated as medicinal products. This means that their processing, storage, distribution and use in patients must respect the requirements of Regulation 1394/2007 on Advanced Therapy Medicinal Products ('ATMP Regulation') as well as all other relevant provisions of the EU medicines rules. This means that their processing, storage, distribution and use in patients must respect the requirements of Regulation 1397/2004 on Advanced Therapy Medicinal Products ('ATMP Regula-

tion') as well as all other relevant provisions of the EU medicines rules (see Sections 20.2 and 22.2).

The following generic chapters from this Guide (see part A) all apply to HSC 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. Processing and storage (Chapter 7);

h. Principles of microbiological testing (Chapter 8);

i. Distribution and import/export (Chapter 9);

j. Organisations responsible for human application (Chapter 10);

k. Computerised systems (Chapter 11);

l. Packaging and labelling (Chapter 12);

m. Traceability (Chapter 13);

n. Biovigilance (Chapter 14).

This chapter defines the additional specific requirements for HSC transplantation.

21.2. Donor evaluation

HSC for transplantation are obtained from living donors; either from the recipient patient in the case of autologous transplantation, or from an allogeneic donor with a matching human leucocyte antigen (HLA). Allogeneic donors can be related or unrelated to the intended recipient. In the case of an unrelated donor, collection can be from healthy

adult donors or through cord blood donations that are stored and delivered by cord blood banks. ABO-incompatible HSC transplantation is possible.

HSC can be obtained from a variety of autologous or allogeneic sources that include bone marrow, peripheral blood stem cells and cord blood.

For evaluation of allogeneic and autologous donors, written criteria (standard operating procedures [SOP]) should exist. Criteria must take into consideration not only the recipient's but also the donor's safety. The risk of donation should be evaluated and documented. To avoid a conflict of interest, the physician who evaluates the donor should be independent of the transplantation team. In the case of HSC from peripheral blood, donor evaluation must be completed before the donor receives the mobilisation agent.

Criteria should include, in addition to general requirements, at least:

a. pregnancy assessment in female donors;

b. adequate cardiac, pulmonary, hepatic and renal function;

c. suitability for anaesthesia (for collection of bone marrow);

d. assurance of adequate venous access;

e. haematologic studies;

f. ABO and Rh typing from two blood samples taken independently (for allogeneic donors);

g. ABO and Rh typing from two blood samples taken independently;

h. screening for red blood cell (RBC) antibody for allogeneic recipients;

i. exclusion criteria;

j. policy for making decisions in cases of 'only one' donor but who do not meet eligibility criteria (e.g. only one suitable donor with risky behaviour).

Donors with history of malignant disease 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.

In exceptional cases, donors with active hepatitis B virus (HBV) infections can be considered suitable for sibling donation in situations of clinical urgency with adequate recipient protection (e.g. vaccination, hyperimmunoglobulin anti-viral therapy, etc.).

Specificity of allogeneic HSC transplantation lies in the fact that, for the vast majority of patients, the HSC graft is infused immediately after collection. Thus, safety is reliant mostly on stringent evaluation of donors that can be undertaken appropriately only if anticipated widely.

The increasing age of recipients of allogeneic HSC transplants is related to the increasing age of the population. Even if the age limit to donate is well-defined for unrelated donors and is under 55–60 years for most international registries, these limits do not strictly apply for related donors. The decision process to collect from elderly donors must include an accurate risk assessment.

Some elderly donors will present with comorbidities (discovered or not) during evaluation. If these comorbidities result in contra-indication of the person for HSC donation, the physician who decided to contraindicate this person must ensure that a correct medical follow-up will be made in accordance with the medical condition of the rejected donor.

If the comorbidities found during evaluation allow HSC donation, the physician in charge must manage these comorbidities during the entire donation process, including specialist consulting as needed.

Transplantation programmes should plan in advance on these proposed guidelines to care for elderly donors in whom comorbidities are identified during recruitment. Such planning can be achieved only through anticipation of the recruitment and medical validation of the donor and the entire transplantation procedure.

Recently, 'haplo-identical' allogeneic transplantation protocols have been used increasingly for patients lacking a fully matched donor (related or unrelated). In parallel, numerous cord blood-unrelated mismatched transplantation protocols are used widely within transplantation programmes.

Thus, indications for 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 under intense investigation and should be evaluated in the context of biomedical research or registry studies. To date, results of prospective clinical studies comparing all sources of alternative HSC donors (especially cord blood *versus* haplo-identical) are lacking. Hence, transplantation programmes should carefully follow their own 'local' guidelines in defining the 'best donor' for each patient in each situation.

Particular attention should be paid in family-mismatched donor selection (parents, brothers/sisters, adult children, others). The criteria of choice should be explained in advance to the patient and potential family donors.

21.2.1. Specific considerations for paediatric donors

It is ethically permissible for children to be donors if the following criteria are fulfilled [1]:

a. There is no medically equivalent histocompatible adult relative who is willing and able to donate.

b. There is a strong personal and emotionally positive relationship between the donor and recipient (practically only for a sibling).

c. There is some likelihood that the recipient will benefit from transplantation.

d. The clinical, emotional, and psychosocial risks to the donor are minimised and are reasonable in relation to the benefits expected to accrue to the donor and recipient.

e. Parental permission and, if appropriate, child assent have been obtained.

If minors are being considered as HSC donors, in addition to the criteria shown above, national regulations should be followed.

Children should become donors only in very specific circumstances and never through public registries (see Chapter 3).

21.3. Collection

Most HSC are collected using two technologies: collection of bone marrow and apheresis. The advantages and disadvantages of these technologies are shown in Table 21.1.

Table 21.1. Advantages and disadvantages of methods of HSC collection [2]

Collection method	Advantages	Disadvantages
Bone marrow	• Single procurement; • No need for a catheter; • Use of cytokines not necessary.	• Requires general or epidural anaesthesia; • Slower engraftment of neutrophils and thrombocytes; • Higher risks of morbidity and mortality; • Potential contamination with skin contaminants; • Potential need for blood transfusion; • Potential contamination with tumour cells in autologous HSC collections.
Peripheral blood	• Does not require anaesthesia; • Faster engraftment of neutrophils and platelets; • Associated with lower rates of morbidity and mortality; • Potentially less contamination of product by tumour cells.	• Collection may take several days (i.e. several procedures may be needed); • Sometimes requires placement of a large-bore, double-lumen catheter for collection; • Haemorrhage, embolism, pneumothorax/haemothorax and infection are possible complications related to insertion of a central venous catheter; • Risks associated with mobilisation agents.

For HSC collection, written procedures must be established.

Severe adverse reactions can occur in donors during and after allogeneic HSC donations. Hence, careful training of clinicians caring for donors is needed, as is appropriate follow-up of donors [3–7].

21.3.1. Bone marrow

Collection of bone marrow is an aseptic process that should be undertaken in an operating theatre by appropriately trained personnel. Special attention should be paid to the training of clinicians in bone marrow collection and after-care of the donor and to vigilance and surveillance of donors as well as of recipients. Provisions for counselling of donors and their routine post-donation follow-up must be provided.

Bone marrow for therapeutic use is obtained through multiple punctures, usually of the posterior iliac crests. When absolutely necessary, the anterior iliac crests can also be used. The sternum is not recommended for purposes of bone marrow collection. Punctures are usually undertaken under general anaesthesia. Epidural anaesthesia may be considered. A pre-anaesthesia visit of the donor is mandatory before collection of bone marrow.

For collection of bone marrow, written procedure(s) should be established, elaborating at least:

a. preparation of media used to obtain bone marrow;

b. provision of containers for collected bone marrow;

c. monitoring of the volume of the collected bone marrow;

d. irrigation of collection syringes;

e. filtering the bone marrow.

Bone marrow HSC grafts contain bony spiculus, fat and clots that should be filtrated during collection or processing. The bone marrow total nucleated cell number (TNC) is used to determine the adequacy of the collection. The recipient's body weight and type of post-collection manipulation determines the target TNC and volume of bone marrow to be collected. Usually, it is sufficient to collect 10–15 ml of

bone marrow/kg recipient weight, resulting in total volumes of 1000–1500 ml. Usually, 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 $2–3\times10^8$ TNC/kg recipient body weight.

Bone marrow HSC grafts have high haematocrits and can contain ≤450 ml of RBC. Hence, donors must be monitored appropriately and treated for iron deprivation after bone-marrow collection as part of routine care and follow-up. Adverse reactions associated with bone-marrow collection is associated with anaesthesia, pain at aspiration sites, bruising and, rarely, local infection.

A 24-h blood component donor support protocol, including the provision of cytomegalovirus (CMV) antibody-negative (or equivalent), irradiated and leucocyte-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. Donation of autologous RBC before collection can be considered but should take into account potential induction of iron deficiency because the time from final selection of donor to collection can be short. Donation of autologous blood should be over a reasonably long period before collection but ≥72 h [8]. Autologous blood must be taken in a blood-collection facility that meets applicable national/international requirements.

Collection procedures in paediatric donors shall be adjusted according to age and size [9].

21.3.2. Peripheral blood

HSC from peripheral blood should be collected in a therapeutic apheresis facility by personnel who have appropriate experience in care for haematology or oncology patients, HSC mobilisation and therapeutic apheresis. Special attention should be paid to paediatric patients and the special circumstances pertaining to apheresis in young patients, whose weight (usually <20 kg) places them at risk of haemodynamic changes, both on commencement and during the procedure. Exper-

tise to carry out apheresis is of particular importance for low-weight children (<20 kg) for autologous collection (which is usually indicated in solid tumours or haematologic malignancies); the transplantation programme must maintain trained and experienced personnel for apheresis in paediatric units.

Mobilisation of HSC to peripheral blood before autologous collection is ensured by administration of various types of mobilisation regimens. Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is the usual haematopoietic growth factor used to mobilise progenitors. RhG-CSF can be administered alone or in combination with chemotherapy in mobilisation of autologous donors or other agents (e.g. plerixafor, immunostimulants).

Circulating levels of CD34+ cells guide commencement of apheresis. The number of cells required varies with the size of the patient/recipient and number of transplantations indicated (double grafting is indicated for some diagnoses). Collection centres should have protocols that can determine the optimal number of cells to be collected taking into account the patients' wellbeing during and after collection, as well as their needs as future recipients.

Cell mobilisation is initiated using rhG-CSF before allogeneic donation from healthy adult donors. Immediate and severe side effects associated with rhG-CSF administration are scarce and raise the issue of inpatient administration *versus* outpatient administration. Donors who live far away from the transplantation centre will require administration at home. The transplantation programme or the physicians in charge of mobilisation and collection of HSC from the donor should be informed in detail of the necessary measures to be taken in case severe adverse reactions (SAR) occur, especially for anaphylactic shock, spleen rupture, capillary leakage and acute hepatitis.

Table 21.2. Very common adverse reactions associated with HSC mobilisation (>10%) [2]

Agent	Adverse reaction
Filgrastim	Musculoskeletal pain
Lenograstim	Thrombocytopaenia
	Hyperleucocytosis
	Transitory elevation of levels of liver enzymes
	Elevation of levels of lactate dehydrogenase
	Headache
	Asthenia
Plerixafor	Diarrhoea
	Nausea
	Reaction at infusion site

In the selection process of the mobilisation agent, the World Marrow Donor Association document *Concerns about the use of biosimilar granulocyte colony-stimulating factors for the mobilization of stem cells in normal donors: position of the World Marrow Donor Association* [10] should be consulted. The relevant mobilisation agent should be used in accordance with the latest approved Summary of Product Characteristics.

Before each apheresis procedure, the donor (autologous and allogeneic) shall be monitored. At least the following studies should be carried out:

a. complete blood count (including platelet count) ≤24 h before collection;

b. haemoglobin and haematocrit;

c. vital signs and temperature;

d. update of medical history.

Collected apheresis volumes vary depending on the collection protocol and cell separator. The target collection 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 similar but, because of the longer time to engraftment of neutrophils and platelets associated with allogeneic transplantation, HSC doses above 4×10^6 CD34+ cells/kg might be needed. In addition to optimising HSC collection, apheresis should ensure that collected cells have minimal contamination with mature cells that could compromise subsequent processing steps or contribute to side-effects in recipients. HSC from apheresis contain small volumes of RBC (haematocrit <5%) so the risk of donation-related anaemia is very low [11].

Targeted cell dose could be reached in one or more apheresis procedures.

Some Health Authorities do not permit the use of rhG-CSF in paediatric donors and so other collection strategies may be employed such as bone marrow donation.

Procurement for donor lymphocyte infusions (DLI) is detailed in Chapter 22.

21.3.3. Cord blood

The Council of Europe has been studying the issue of cord blood donation for several years and has always been concerned about the proliferation of private cord blood banks dedicated to the collection and storage of cord blood for autologous use. This concern resulted in adoption of Recommendation Rec (2004) 8 of the Committee of Ministers to member states on *Autologous cord blood banks* and its

explanatory memorandum [12], which recommends that member states allow establishment of cord blood banks only for altruistic and voluntary donations of cord blood. The recommendation also states that the promotion of cord blood donation for autologous use and the establishment of cord blood banks for autologous use should not be supported by member states or their health services. Where autologous cord blood banks are established, the promotional material or information provided to families must be accurate, and fully informed consent to cord blood storage must be obtained. For this purpose, the Council of Europe has produced the brochure *Umbilical cord blood banking. A guide for parents*, to provide clear, accurate and balanced information about the use of cord blood in medical treatment and to guide parents through their blood storage options [13].

Autologous (or family use) cord blood banks must clearly inform parents about the differences between the different medical objectives of autologous and allogeneic donations and about the uncertainties relating to the medical applications of autologous cord blood preservation. In any case, autologous cord blood banks must meet the same quality and safety standards as for allogeneic cord blood donation and banking.

Umbilical cord blood can be obtained at birth from fully informed women who have previously consented to allogeneic donation (including related donations), following normal delivery of a healthy baby. No modification in delivery practices, with a view to increasing the volume of cord blood collected, is allowed. Collection after caesarean delivery is possible, provided that the baby and mother are well. Collection should be carried out only by trained staff.

Because the collected cell number is a critical parameter used by transplant teams to select umbilical cord blood, it is important that blood volume collection be maximised within the confines of not interfering with normal delivery practices.

Traceability of the collected cells from the mother and the newborn must be ensured by personnel that collect the blood. Systems to allow reporting on the health of the mother and newborn subsequent to

donation of umbilical cord blood are part of the umbilical cord blood banking process.

21.4. Processing

Processing of minimally manipulated HSC is intended to provide appropriate conditions for preservation and storage or to improve the risk–benefit ratio of autologous or allogeneic HSC transplantation [9, 11]. It does not affect the main biological properties of the collected 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 Chapter 7.

Specificities of processing HSC with regard to air-quality requirements are shown in Table 21.3.

Within the EU, tissues that are exposed to the environment without a subsequent microbial inactivation process should be processed in environments with an air quality equivalent to those of Grade A, as defined in EU Good Manufacturing Practice (GMP), with a background environment at least equivalent to EU GMP Grade D.

Taking the factors detailed in Table 21.3. into consideration, it is appropriate that processing of HSC takes place in a microbiologically and climate-controlled environment (control of temperature, humidity, ventilation, air filtration) with validated cleaning and disinfection and with Grade D air quality as background. The same requirements apply for autologous or allogeneic donations.

Table 21.3. Factors influencing the specification of processing air quality for haematopoietic stem cells

Criterion	Haematopoietic stem cells-specific
Risk of contamination of tissues or cells during processing	Cryopreservation or purging of HSC is mostly closed. Sterile barrier can be compromised in a moment after adding cryoprotectant, monoclonal antibodies or other solutions by sterile needles.
Use of antimicrobials during processing.	Use of antimicrobial agents during HSC processing is not applicable. Nevertheless, in some cases, even HSC 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. Samples can be taken from the cells or residual components depending on the product volume. Procedures for microbiological detection shall 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 this tissue 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. The main factor is the thawing procedure. Thawing in a water-bath is associated with a significantly higher risk of contamination compared with a dry thawing method.

21.4.1. Volume reduction

Volume reduction is either a preparatory step to further processing (including cryopreservation and storage) or a means to reduce the volume of the infused cells and, thus, prevent recipient side-effects relating to volume overload in the transplanted patient. Various centrifugation-based techniques can be used. Cell loss associated with volume reduction must be evaluated and expected recoveries defined.

21.4.2. Red blood cell depletion

Red cell depletion is a critical step in cases where there is major ABO incompatibility between a donor and a recipient in the allogeneic setting (HLA identity does not preclude the existence of major or minor ABO incompatibility). Various techniques for blood cell depletion are available, including buffy-coat centrifugation or apheresis cell separation. The efficiency of the technique must be monitored by measuring the residual content of red cells which should be as low as possible. Similarly, the cell and progenitor loss associated with such procedures must be evaluated and expected recoveries must be defined.

21.4.3. Plasma removal

Plasma removal represents a critical step in cases with minor ABO incompatibility between allogeneic donor and recipient setting (HLA identity does not preclude the existence of major or minor ABO incompatibility). The necessity of plasma removal in case of minor ABO incompatibility can be judged using a titration of anti-A and anti-B antibodies in the donor during the period that precedes the collection of the donation, Plasma removal is usually done by centrifugation of the collected cells. The cell loss associated with such procedures must be evaluated and expected recoveries must be defined.

21.4.4. Cryopreservation, thawing and infusion

21.4.4.1. *Cryopreservation*

The purpose of cryopreservation is to preserve HSC in such a way as to ensure their viability and potency. To minimise the volume infused, peripheral blood and bone marrow may be concentrated before cryopreservation. The volume of cell suspension per bag should be adjusted by the body weight of the patient and maximal daily dose of dimethyl sulfoxide (DMSO) (1 g/kg body weight). Special attention should be paid if the recipient is a small child.

Cryopreservation is used systematically in the autologous setting and by cord blood banks that prospectively collect and store umbilical

cord blood. In the allogeneic setting, cell collection from the donor is usually synchronised with administration of a conditioning regimen to the recipient. Allogeneic donations are usually infused into the recipient within hours of collection of the donation. However, an increasing proportion of allogeneic HSC are being cryopreserved for logistical reasons: the donor living far from the transplantation centre; international exchange of donations; professional constraints; unforeseen changes in transplantation schedules.

The cryopreservation method for HSC collected from peripheral blood, bone marrow or blood from the umbilical cord is the same. The method involves addition of 5–10% DMSO in a HSC suspension in a protein-rich medium with or without dextran or hydroxyethyl starch (HES). Immediately after DMSO addition, HSC are cooled at –1°C to –2°C per minute. Freezing takes place in an apparatus in which vapour-phase liquid nitrogen is pumped into the freezing chamber. Freezing machines require manual supplementation by an operator carefully observing the temperature of the solution, or are controlled by a microprocessor. The final phase of cooling is usually quicker, with the temperature drop adjusted to 5 °C/min. When the mixture has reached approximately –120°C it is transferred to a storage container.

Once frozen, HSC can be stored in vapour-phase liquid nitrogen or in liquid nitrogen at below –130 °C. Variations in cryopreservation methods include the concentration of frozen cells, amount and source of plasma protein, and the cooling rate. The method chosen must be validated.

Maximal shelf-life has not been defined for HSC from the bone marrow and peripheral blood. Bone marrow HSC have been transplanted successfully even 11 years after cryopreservation.

21.4.4.2. *Thawing and infusion*

Cells can be thawed at the bedside or in a stem cell laboratory. HSC should thawed at 37 °C at a heating rate of 100 °C/min. HSC must be washed after thawing to remove most of the DMSO.

The risks of washing are significant cell loss, decreased viability, and decreased chance of engraftment. Hence, washing of HSC 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.

HSC should be infused immediately after thawing at 5–20 ml/min, although this interval may be longer if the HSC are washed. HSC should be infused using a standard transfusion set. Leucoreduction filters must be avoided.

21.4.4.3. *Non-frozen storage*

Progressive loss of HSC occurs during non-frozen storage. Nevertheless, HSC should be stored in non-frozen conditions before processing and infusion or during transportation. Survival of HSC stored in a non-frozen state is dependent upon the concentration of cells, buffering capacity of the solution, the chemicals added, collection volume, type and the gas-diffusion capacity of the storage bag, and storage temperature. Cell viability decreases and the risk of microbiological contamination increases during storage at room temperature as well as in refrigerators. Therefore, maximum storage in the non-frozen state should be ≤72 h.

The processing facility should undertake a validation study of the storage condition.

21.4.5. Cell selected preparations

Several clinically applicable immuno-affinity methods for positive selection of CD34+ cells have been developed. A limited number of Conformité Européenne (CE)-marked automated instruments can be used to capture CD34+ cells from bone marrow, peripheral blood or umbilical cord blood on the large scales needed for clinical transplantation. The use of such medical devices requires specific training for personnel involved in these procedures.

21.4.5.1. *T-cell depletion in the allogeneic setting*

T-cell depletion prevents or reduces the risk of GvHD after allogeneic transplantation from related or unrelated donors, but is rarely used for HLA-identical transplantation from living donors or from umbilical cord blood. This is because the advantages of reducing GvHD are offset by associated increases in relapse rates and graft failures. Indications for T-cell depletion remain limited to specific forms of allogeneic transplantation, such as from haplotype-mismatch donors and following myelo-ablative conditioning regimens. 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 (usually 1×10^5 CD3+ cells and 1×10^5 CD19+/CD20 cells/kg of recipient weight) and their guidance sought if the set objective is not met.

21.4.5.2. *Tumour cell depletion in the autologous setting*

Autologous tumour cells collected with normal HSC 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 has been practically abandoned but, if a transplant team decides to use such a procedure, then tumour cell depletion should be as extensive as possible and then accurate determination of the residual tumour cell content is crucial, using either immuno-histochemical techniques, flow cytometry or molecular biology techniques.

21.4.6. **Depletion of allo-reactive immune effectors**

Total T-cell depletion is associated with positive (i.e. GvHD prevention) and negative (i.e. prolonged immunosuppression) consequences that prevent its adoption in routine clinical practice. Other specific procedures evaluated by clinical trials include depletion of activated and allo-reactive 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. It is important that T-cell depletion be as extensive as possible. Therefore, accurate determination of the residual T- and B-cell content is critical. The highest acceptable dose of residual allo-reactive T-cells must be defined in advance by the medical team in charge of the recipient and their guidance sought by the collection team if this objective cannot be met.

21.4.7. Immunocompetent cells used after haematopoietic stem cell transplantation

To enhance immune responsiveness after HSC transplantation, the following cells are being used and/or evaluated in clinical trials:

a. alloreactive donor T-cells (donor lymphocyte infusions) for prophylaxis and treatment of relapses after HSC transplantation

b. T regulatory cells for the prevention and control of GvHD

c. natural killer (NK) cells as graft *versus* leukaemia (GvL) effectors by alloreactivity of KIRs in donor-recipient direction

d. human cytomegalovirus (CMV)-specific T-cells ($CD4^+$ and $CD8^+$ cells) for the treatment of CMV infection

e. Vaccination with peptide-loaded dendritic cells (DC) for induction of tumour-specific T-cell responses for the treatment of metastatic disease transplantations, treatment of GvHD

f. Mesenchymal stem cells enhance engraftment in allogeneic and autologous HSC transplantations; treatment of GvHD

In case the cells are subjected to substantial manipulation (including expansion or stimulation), these cells fall under the definition of ATMP in EU member states (see Sections 20.2 and 22.2).

21.5. Quality control

21.5.1. 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.

21.5.2. Safety controls

Detection of transmissible infections is undertaken through donor screening (using microbiological 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 collection, processing and distribution.

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 sub-populations of leucocytes in the collected cell preparations must be measured. High numbers of mature cells such as granulocytes may negatively impact on several subsequent processing steps, and may contribute to recipient side-effects at re-infusion.

Removal of red blood cells from ABO-incompatible allogeneic cell preparations through specific processing procedures must be documented when relevant, as must the removal of T-cells or other immune effectors from allogeneic cell preparations through specific processing procedures.

The removal of tumour cells from autologous cell preparations using specific processing procedures must also be documented where appropriate.

21.5.3. Quality controls (including potency assays and markers)

The number of total nucleated cells (TNC) remains a widely-used measure for evaluating the quality of collected bone marrow. The cell dose for recipients is usually expressed in TNC/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.).

The proportion of cells of the erythroblastic lineage may be high in umbilical cord blood and must be documented. CD34+ cell counts are used as a surrogate marker for HSC, both in the peripheral blood of individuals undergoing mobilisation regimes and in the collected cells, whether from apheresis following mobilisation or from umbilical cord blood donation. The value of CD34+ cell counts for assessing the MRA of bone marrow is less well established; in part, due to the near abandonment of autologous bone marrow transplantation and the decrease in the proportion of allogeneic HSC transplantation undertaken with bone marrow. CD34+ cell counts are usually measured by flow cytometry, using monoclonal antibodies that recognise one or several epitopes on the human CD34 membrane antigen. It is recommended to use diagnostic kits rather than a combination of reagents. Diagnostic kits include all the necessary reagents to produce a measure of the percentage and absolute number of CD34+ cells in biological samples, and ensure that detected CD34+ cells are alive and belong to the progenitor cell compartment (as opposed to pro-erythroblasts). Use of a single-platform, rather than a dual-platform, minimises errors in calculating cell counts. For example, the International Society for Hematotherapy and Graft Engineering (Interna-

tional Society for Cellular Therapy) algorithm provides a robust and reproducible gating strategy to measure CD34+ cells.

Potency assays for cell preparations used for autologous or allogeneic transplantation rely on the detection of colony-forming units (CFU) in clonogenic assays, using semi-solid media. These functional tests are hampered by the delay required to produce results (usually two weeks). Thus, the results are usually only available long after a non-cryopreserved cell preparation has been transplanted in an allogeneic recipient. Clonogenic assays are hampered by poor intra- and inter-laboratory reproducibility. This particular issue could be improved by using commercially available and standardised culture media and by participation in proficiency testing and external quality assessment schemes. It remains to be demonstrated that the recent introduction of image analysers can further improve the situation. Colony-forming units: granulocyte/monocyte (CFU-GM) numbers can be interpreted in two ways: the absolute number of clonogenic progenitors per kilogram of recipient weight can be used as a surrogate marker for MRA (but this has largely been supplanted by CD34+ cell counts), or the frequency of CD34+ cells that form colonies in culture can be used to provide additional information about the functionality of the graft. In the latter case, a policy should be defined to deal with cases where CD34+ cells clone at a low frequency. Some national regulations and guidelines define in which context CFU-GM measurements are mandatory. As a minimum, it is recommended that CFU-GM measurements should be carried out during inspections and proficiency testing exercises in order to demonstrate the ability of the tissue establishment to prepare functional haematopoietic stem cell preparations.

The same quality standards apply for autologous and allogeneic donations.

21.5.4. Release criteria

The cell processing facility, along with its clinical counterparts, must define which safety and quality controls serve as release criteria. It

must also define which criteria must be strictly met and which ones may lead to documented waivers. Specific instructions should be established in the tissue establishment on how to deal with the recipient, donor and stem cells throughout the donation and processing and issue stages and all the way through to transplantation.

Processing and transplant facilities should agree on the dose (nucleated cell count, mononuclear cell count, $CD34^+$ cell count and/or clonogenic assays as appropriate for the source of HSC) required to achieve reliable and sustainable engraftment. For cord blood, the volume of the collection correlates to the dose and must also be measured accurately. In donation of bone marrow, the maximum volume of donation, calculated for each individual donor, should be agreed on a national basis.

If cells are required for administration to a patient, a prescription for infusion is generated. This prescription lists the cell preparations that are suitable for that patient. It is usually accompanied by a copy of the protocol flowchart, which provides specific information on dosing. If necessary, the cells may be manipulated before infusion and this is recorded on a worksheet and activity report.

21.6. Storage

Storage must be done in conditions that minimise the risk of cross-contamination.

Conditions for temporary storage must be defined for each type of cell preparation and for each of the stages of the process, from collection to pre-processing and post-thawing, etc.

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.

Internal and external transport must be controlled. Transportation within the same institution (i.e. from the collection facility to the processing facility or from the processing facility to the transplant ward,

etc.) must be defined by standard operating procedures. When service providers are used for transportation of a fresh 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 the cell preparations. This includes appropriate training of the personnel in charge of transportation.

21.7. Packaging and labelling

Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect personnel and the environment. The primary packaging must be made of a biologically compatible material. Cryopreservation requires that low-temperature-resistant packaging is used, which can also withstand contact with liquid nitrogen.

Labelling must unambiguously identify the donor, the intended recipient, the cell preparation and its nature as well as the additives used and the conditions under which the cells are to be stored and distributed. Following procurement, the donor identifier should be on the 'transit' label when tissues are distributed to the tissue establishment for processing. The recipient must be identified (but not the donor) when tissues are distributed for end use. In all cases there must be an audit trail to the donor.

International standards for labelling now exist (e.g. ISBT 128, European coding system, etc.) and must be used to promote consistency, traceability, aid international exchanges and facilitate vigilance and surveillance.

21.8. Vigilance and surveillance

For an effective vigilance and surveillance (V&S) system for tissues and cells used in transplantation and assisted reproduction, the European Union project SOHO V&S was developed in 2013. *Guidelines on Vigilance and surveillance of human tissues and cells* [14] were published for healthcare professionals responsible for all types of HSC

(bone marrow, peripheral blood stem cells, cord blood) for human application.

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 adverse reactions and events (SARE). General guidance on implementation of good V&S practice as well as definitions of SARE is described in Chapter 14.

Tissue establishments should have written procedures for managing SARE. They should also provide centres carrying out clinical applications with accurate and updated information on various SARE in the area of HSC transplantation.

21.8.1. Serious adverse reactions in the recipient

21.8.1.1. *Complications related to haematopoietic stem cell infusion*

Infusion of HSC is, in general, well tolerated. Complications are consequences of immunological incompatibility, iatrogenic toxicities, microbiological contamination and manufacturing/administrative errors. Some complications are similar to complications caused by transfusion of standard blood products, which is particularly true for allogeneic HSC preparations if they contain ABO-incompatible RBC or plasma. Other complications are specific to HSC infusion, and are related to allogeneic and autologous HSC.

Adverse reactions can be immunological and non-immunolological, acute and delayed.

21.8.1.1.1. *Haemolysis of red blood cells*

Donor–recipient mismatching in erythrocyte antigens is not a contraindication for HSC 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 HSC preparation because the content of RBC and plasma is different.

Acute haemolytic reactions are severe complications of HSC infusion. They are caused by ABO incompatibility between the donor and recipient. Usually, the risk of acute haemolysis is greater if the RBC 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 the same concentration of RBC as whole blood and can cause haemolysis. The risk of acute 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 a HSC 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 HSC 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 HSC preparation that can produce antibodies against the recipient's ABO or other RBC antigens, the haemolysis can be more serious. At greater

risk are recipients who receive ABO minor-incompatible HSC. Typically, haemolysis will occur within 1–3 weeks after HSC infusion. Occasionally, life-threatening haemolysis can occur. Apheresis HSC 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 HSC are used, passive haemolysis is more common.

21.8.1.1.2. *Complications within the respiratory tract*

HSC infusion frequently induces complications within the respiratory tract. During administration, patients often start coughing. Coughing is related primarily to application of cryopreserved autologous HSC, 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 neutrophil antibodies or bioactive mediators cause granulocyte activation in the pulmonary vasculature, 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 HSC 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 HSC 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.

21.8.1.1.3. *Febrile non-haemolytic reactions*

During HSC infusion, patients may experience febrile non-haemolytic 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 with chills, rigor and mild dyspnoea without evidence of haemolysis. This phenomenon may reflect the action of antibodies against leucocytes 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 HSC 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.

21.8.1.1.4. *DMSO toxicity*

DMSO is the most widely used cryoprotectant but 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 HSC.

Within minutes of starting the infusion a metabolite of DMSO, dimethyl sulfide, 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 a 1 g/kg, which is equivalent to an infusion of 10 ml/kg of cells in 10% DMSO solution. Premedication with antihistamines, slowing the infusion rate, increasing the resting time between multiple infusion aliquots, dilution of thawed HSC 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.

21.8.1.1.5. *Neurological complications*

Neurological symptoms during HSC infusion varies widely. Headache is common and can be related to increase intravascular volume. Occasionally, patients experience more severe side-effects such as muscle spasms and seizures. Cerebral infarcts and acute encephalopathy are rare. Simple muscle spasms often resolve spontaneously. For patients with acute mental changes, loss of consciousness or seizures, urgent intervention is necessary. HSC infusions should be stopped; rapid neurological assessment should be done as well as basic laboratory tests, including electrolytes and glucose. If seizures persist, anti-epileptic drugs are indicated. Patients should be transferred to an ICU.

Neurological complications are probably caused by DMSO, but this suspicion has not been clearly demonstrated.

21.8.1.1.6. *Cardiac toxicity*

Cardiac toxicity is common and manifests as bradycardia and other disorders of cardiac rhythm. It is usually mild (though severe cases of arrhythmias have been reported). Severe bradycardia occurs more often in recipients of cryopreserved bone marrow, and may require aggressive supportive care.

Aetiology may be because of hypervolaemia due to extensive hydration before infusion, large volume of transplant, hyperosmolality of DMSO, hypothermia, lysis of graft cells and underlying cardiac conditions.

21.8.1.1.7. *Allergic reactions*

Allergic reactions usually manifest as urticaria and pruritis. Most occur in patients receiving allogeneic transplants. Anaphylactic-type reactions are rare. Allergic reactions present as bronchospasm and/or laryngospasm, hypotension, severe dyspnoea, pulmonary and/laryn-

geal oedema, facial burning and flushing, abdominal pain, diaphoresis, diarrhoea and dizziness.

Causes of allergic reactions are not clear. They may be related to the substances used during cell collection, cell processing or cryopreservation, such as HES or DMSO, that can react with antibodies in the donor or recipient plasma, or with anti-immunoglobulin (Ig) A antibodies in IgA-deficient recipients. No laboratory tests can help to predict or prevent allergic reactions. Therapy is dependent upon symptoms. For mild reactions, administration of antihistamines will be helpful or, in severe cases, corticosteroids, epinephrine and cardiorespiratory support.

21.8.1.1.8. *Anticoagulation effects*

Patients receiving non-cryopreserved and non-manipulated bone marrow have a greater risk of haemorrhage because of high concentrations of unfractionated heparin in bone-marrow grafts.

Risk of bleeding is increased in thrombocytopaenic recipients.

21.8.1.1.9. *Hypertension/hypotension*

Hypertension is more common in cryopreserved HSC, particularly if collected by apheresis because such grafts have a higher volume. Hypertension is a result of acute volume overload due to rapid infusion, prophylactic hydration and the hyperosmolality of the infused preparation.

Hypotension is also more common in cryopreserved HSC. It is linked with vasodilatation due to histamine generation. Premedication by anti-histamines decrease the incidence and severity of hypotension.

21.8.1.1.10. *Acute renal failure*

Acute renal failure is more common in application of cryopreserved bone marrow. It is caused by a large volume of poorly preserved and haemolysed RBC and cellular debris.

21.8.1.1.11. *Bacterial contamination*

Bacterial contamination of an HSC product is possible. Bone marrow, which is collected into an open system, has a higher rate of contamination than HSC collecting from peripheral blood. Contamination may occur at several steps in the process. It can be due to occult asymptomatic bacteraemia in the donor. Because of the particular nature of the graft and the recipient's condition due to the treatment, for autologous donation, it is vital that HSC are collected irrespective of the possible 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 HSC can also occur during collection, processing, storage, thawing or sampling due to an interruption of sterile methods.

After transfusion of contaminated HSC, 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 to bacterial septicaemia. If such symptoms occur during HSC administration, the infusion should be stopped immediately and all infusion bags and equipment examined.

Known bacterial contamination of an HSC unit is not an absolute contraindication for HSC 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.

21.8.1.1.12. *Transmission of infectious diseases*

HSC preparations should be tested for transfusion of transmittable 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 HBV, hepatitis C virus (HCV) and human immunodeficiency virus (HIV). For other viral infections the period will differ depending on the incubation period.

All cases of suspected post-transplantation infection must be reported immediately to the collection site or donor registry.

21.8.1.1.13. *Engraftment failure*

After HSC transplantation, recovery must occur in populations of myeloid, erythroid and immune cells. 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 later. Engraftment is dependent upon the dose of progenitor cells, method of cell preparation, function of bone-marrow stroma, intensity of the preparative regimen, and donor–recipient relationship.

Measurement of granulocyte and platelet engraftments provides essential information about the success of clinical protocols as well as the quality of collection and processing of HSC.

Primary graft failure for HSC transplantation from bone marrow or peripheral blood is defined as a lack of neutrophil engraftment 28 days after transplantation. For HSC transplantation from cord blood, the time point for primary graft failure is 42 days after transplantation. Leucocyte recovery is designated as the first of three successive days in which the absolute neutrophil count is $>500\times10^6$/L. Platelet engraftment is designated as the first day in which the platelet count is $>20\times10^9$/L in an untransfused patient. The sign of erythroid recovery is $>30\times10^9$/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 compatibility, ABO incompatibility or other reasons that can be attributed to the patient.

The Transplant Unit should report graft failure to the tissue establishment to enable thorough investigation of the quality and handling of grafts.

21.8.1.1.14. *Graft versus host disease*

GvHD is a serious and potentially lethal complication of allogeneic HSC transplantation. GvHD occurs if infused T lymphocytes engraft in the recipient and react against the recipient's tissues. Any allogeneic HSC preparations can cause GvHD. Acute GvHD occurs <100 days after transplantation, whereas chronic GVHD occurs >100 days after transplantation. Risk factors for the development of GVHD are: donor–recipient relationship (HLA disparity, gender matching, donor parity, donor age, ABO group mismatching), stem cell graft factors (source and graft composition, cell processing) and transplantation factors (condition and post-transplant immunosuppression regimens).

Clinical manifestations of GvHD typically involve the skin, liver and gastrointestinal tract, but can affect the eyes, oral mucosa, vagina, lungs and neurological system.

To predict the outcome of acute GvHD, scoring based on organ involvement is important. Chronic GvHD is more likely in recipients of peripheral HSC than in recipients of bone-marrow HSC. It can be localised, affect only skin areas, and manifest as progressive systemic sclerosis, Sjögren's syndrome or primary biliary cirrhosis.

Treatment of GvHD includes high-dose corticosteroids, T-cell-suppression drugs and monoclonal antibodies targeting T-cells.

21.8.2. Serious product adverse events/reactions

In the case of serious adverse events (SAE) such as inappropriate transportation, receipt of a wrong unit, receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in hospital, infusion of a unit into the wrong recipient, then the transplantation centre must report this matter to the tissue establishment immediately and, according to national legislation, to tissue- and cell-competent authorities.

21.8.3. Serious adverse reactions in haematopoietic stem cell donors

Bearing in mind that HSC donations are voluntary and altruistic acts of assumedly perfectly healthy individuals, it is the ethical and professional obligation of medical professionals and good practice to notify, document, investigate and report SAR in the living donor, ad not only those influencing the quality and safety of tissues and cells. SAR are uncommon in healthy donors and rare types of SAR or emerging trends are likely not noticed at the national level. SAR in stem cell registry donors are followed at the international level by the World Marrow Donor Association (WMDA). Unfortunately, no such follow-up exists at present for related donors.

21.8.3.1. *Complications in bone-marrow donors*

Donation of bone marrow is, in general, a safe and well-tolerated procedure, but some mild symptoms related to induction of general anaesthesia are common. In most cases, donors recover fully within 2 weeks.

Reactions in bone-marrow donors include constitutional symptoms such as nausea, vomiting, anorexia, insomnia, and fatigue (most common).

Complication related to puncture of bone marrow such as pain upon collection, walking, sitting and climbing stairs as well as minor infection are rare.

Cytopaenias (anaemia, thrombocytopaenia) and more serious reactions such as deep-vein thrombosis (DVT), thromboembolism, cerebrovascular accident and subdural bleeding have also been documented. Post-donation septicaemia and anaesthesia-related complications have also been described, as well as respiratory complications such as pulmonary alveolitis and oedema.

21.8.3.2. *Complications in apheresis haematopoietic stem cell donors*

Complications in apheresis HSC donors are related to apheresis and administration of granulocyte-colony stimulating factor (G-CSF). Symptoms related to citrate infusion are the most common.

Additional complications related to apheresis include haematoma, arterial punctures delayed bleeding, pain from injury to nerves or tendons, thrombophlebitis, local allergy, generalised allergic reactions, vasovagal reactions, haemolysis, and air embolism. All severe conditions requesting hospitalisation or intervention, or death <24 h after collection, should be reported immediately.

Use of G-CSF in mobilisation is, in general, safe. Common short-term reactions related to G-CSF are bone pain, headache, myalgia, nausea, vomiting, diarrhoea, fatigue, fever, irritation at injection site, insomnia, and reduced numbers of thrombocytes. Most of these effects are reversible after discontinuation of G-CSF administration. Other rare reactions are splenic rupture, anaphylaxis, thrombosis, gout, iritis, keratitis, autoimmune hyperthyroidism, acute lung injury, capillary leak syndrome, and exacerbation of rheumatoid arthritis.

Reports from long-term follow-up studies in unrelated apheresis HSC donors mobilised with G-CSF demonstrated a similar incidence of leukaemia and other malignancies to those seen in the general population. All malignant diseases in all donors treated with G-CSF should be reported, regardless of the time of occurrence.

21.9. References

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Chapter 22

Other cells

22.1. Introduction

The rapid advances in medical research and treatment models with allogeneic and autologous cells of various kinds and sources is addressed with examples of some, but not all, in this chapter. In order not to jeopardise this development of future cellular therapies, by failure to maintain quality and safety as well as ethical issues, it is important to follow the general quality and safety demands in the previous sections, but also to consider some specifics for these different cells.

The generic chapters of this Guide (see Part A) 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. Procurement (Chapter 6);

g. Processing and storage (Chapter 7);

h. Principles of microbiological testing (Chapter 8);

i. Distribution and import/export (Chapter 9);

j. Organisations responsible for human application (Chapter 10);

k. Computerised systems (Chapter 11);

l. Packaging and labelling (Chapter 12);

m. Traceability (Chapter 13).

In the European Union (EU), these chapters are only applicable for starting materials (tissues and cells) used for the manufacturing of advanced therapy medicinal products (ATMP).

22.2. Important notice for tissue establishments and other operators established in the EU

In the EU, some of these cell preparations described in this chapter will fall under the definition of an ATMP. Such cell preparations falling under the definition of ATMP are governed by specific requirements and procedures, including prior authorisation by the competent authority (see Chapter 20, Section 20.2). When ATMP preparation takes place in the EU or if they 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 products legislation. However donation, procurement and testing of such cells must comply with the requirements in Directive 2004/23/EC.

Any operator intending to process, store, distribute or use cells which may be considered ATMP should seek advice from the national competent authorities. In case of doubt whether a specific activity with cells is regulated under ATMP, a recommendation from the Committee for Advanced Therapies (CAT) can be requested.

Full information about the EU regulatory framework on ATMP can be found at the Internet websites of the European Medicine Association (EMA) [1] and European Commission (EC) [2].

22.3. Allogeneic mononuclear cells (donor lymphocyte infusions)

22.3.1. Donor selection

Donor lymphocyte infusion (DLI) is a procedure in which lymphocytes from the original stem cell donor are given to the recipient following allogeneic haematopoietic stem cell (HSC) transplantation, to augment the anti-tumour immune response or to ensure that the donor stem cells remain engrafted. The goal of this therapy is to induce a remission of the patient's cancer by a process called the graft *versus* tumour (GVT) effect. The donor T-cells can attack and control the growth of residual cancer cells providing the GVT effect.

Mononuclear cells from the HSC donor are collected by apheresis technique, either soon after the stem cell donation or at a later stage. If the collection takes place 30 days or more after the first procedure, the donor should undergo a new medical evaluation for his/her own safety. In addition, the donor should be tested again for infectious diseases to maximise the safety of the recipient.

22.3.2. Procurement

Collection of mononuclear cells by apheresis technique requires special knowledge and should be done in an apheresis facility with personnel experienced in hemodynamic changes. If possible, peripheral veins allowing for sufficient blood flow should be used for venous access but a pre-evaluation of the veins needs to be carried out by qualified personnel. A central catheter should be placed by a specialist and at a location associated with the lowest frequency of complications. Placement of central catheters may not be allowed in allogeneic donors from certain registries. Donors should be informed about potential complications associated with placement of central catheters (pain, bleedings, infections, thrombosis) and an established procedure for quick access to an intensive care unit is required.

In those cases where a surplus of CD34 + cells is collected during the procurement of HSC from peripheral blood (e.g. when the donor

is in another country and performing a second apheresis may be a problem), the same procedure may be used to collect mononuclear cells for future DLI.

22.3.3. Processing

Processing of the mononuclear cells mainly involves adjustment of volume and cell number according to the clinical protocol used. The number of mononuclear cells and specifically the number of CD3+ T-cells can be determined by flow cytometry and further characterisation of subpopulations of T-cells may be undertaken according to special requirements and needs.

Frequently a T-cell dose of 1×10^6 CD3+/ kg body weight of the recipient is the starting dose, and then further treatments with escalating doses may be used. For the preparation of suitable portions to be frozen, the tissue establishment should validate their freezing technique in order to establish the expected level of viable T-cells after thawing.

22.3.4. Quality control

In addition to the microbiological testing of the cells or supernatant of the cells described in Chapter 8, the specific requirements include establishing the absolute number and the frequency of T-cells (CD3+ and/or subpopulations) and cellular viability.

22.3.5. Storage

DLI may be frozen in ampoules labelled with the desired dose, below –150°C (–150 °C to –196 °C) in the liquid or vapour phase of nitrogen or deep freezer with liquid nitrogen back up.

Precautions should be taken to prevent cross-contamination of infectious agents if stored in liquid phase of nitrogen, using double containers.

22.3.6. Biovigilance

The processing of DLI involves several steps where unexpected events that has to be documented and reported, may occur (see Chapter 14).

For instance lower viability of the frozen and thawed DLI than expected or human errors in calculating the dose of T-cells. DLI may still be used but this has to be documented and a risk analysis carried out.

22.4. Virus specific T-cells

For operators established within the EU, see Sections 20.2 and 22.2.

Donor selection

A viral infection in immunocompromised patients after haematopoietic stem cell transplantation is a frequent cause of morbidity and mortality. It is possible to reconstitute the antiviral immunity of the recipient against specific viruses (e.g. cytomegalovirus [CMV], Epstein–Barr virus [EBV] and adenovirus) with adoptive transfer of donor derived virus specific T-cells.

Also, pre-established virus-specific T-lymphocytes from allogeneic human leucocyte antigen (HLA)-typed third-party donors may be used after HLA-mapping and selection of a suitable HLA-match [3]. If such banked virus specific T-cells are to be used, a risk assessment based on the degree of HLA-mismatch and risk of graft *versus* host disease (GvHD) or graft rejection must be considered by a qualified specialist in allogeneic stem cell transplantation.

Donors should be tested for transmissible diseases in accordance with Chapter 5 and in addition presence of antibodies against the specific virus should be determined before assays for specific T-cells are initiated.

22.4.1. Procurement

A sample of heparinised venous blood (30–50 ml) from the stem cell donor is usually enough to select the virus specific T-lymphocytes from the mononuclear fraction of the blood.

22.4.2. Processing

Selection can be carried out using the interferon gamma (IFN-γ) production of the lymphocytes after stimulation with virus specific pep-

tides. After initial selection, the T-cells need to be expanded in culture with re-stimulation at intervals according to a pre-defined protocol. The use of EBV-transformed B-cell lines as antigen presenting cells for re-stimulation is frequently used, but also other protocols using dendritic cells may be appropriate. The expansion of virus specific T-cells may take 2–4 weeks to obtain enough cells with therapeutic effect according the protocol (a minimum of 1×10^7 cells and repeated at 1–2 week intervals). The possibility to have pre-established HLA-typed virus specific T-cells from third-party donors, with well documented specificities and quality requirements in a cell bank would undoubtedly enhance the possibility of therapeutic effect since it may take too long time to expand virus specific T-cells for a given virus once the patient is affected.

Also non-manipulated specific T-cells may be used after selection, but without further expansion. The use of peptide-HLA multimers facilitates the visualisation and isolation of antigen-specific cytotoxic T-cells. CD8+ T-cells that bind multimeric HLA complexes can be isolated to high purity using magnetic beads or FACS sorting. Newly developed multimeric HLA complexes, binding reversibly to the T-cell receptor, offer the opportunity of selecting non-manipulated antigen specific cytotoxic T cells. The transfer of CMV-specific cytotoxic T-cells freshly isolated from peripheral blood might be superior to the *in vitro* expansion and manipulation of T-cells. The *in vitro* expansion may increase the expression of the pro-apoptotic FAS molecule (CD95) and reduce telomere length of specific T-cells, leading to shorter survival of the adoptively transferred T-cells.

22.4.3. Quality control

The specific requirements for release include assays determining their virus-specificity according to pre-established criteria for IFN-γ production that can be quantified using ELISpot assay or flow cytometry. Depending on the defined and validated protocol, the functional assay can be complemented with phenotypic release criteria using flow cytometry. Expected viability after thawing should also be established if the specific T-cells are stored frozen for repeated *in vivo* transfer.

22.4.4. Storage and distribution

Cultured and released cells can be frozen at suitable portions below –150 °C in liquid or vapour phase of nitrogen or deep freezer with liquid nitrogen back-up.

Precautions should be taken to prevent cross-contamination of infectious agents if stored in the liquid phase of nitrogen, using double containers.

22.4.5. Biovigilance/pharmacovigilance

Whenever adverse events occur during the processing, or adverse reactions during application of the T-cells this should be documented and reported (see Chapter 14). As an example, T-cells that do not fulfil criteria for specificity as measured by *in vitro* methods can still be used as they may fulfil biological effect measured *in vivo*.

22.5. Keratinocytes

For operators established within the EU see sections 20.2 and 22.2.

22.5.1. General introduction

The 'gold standard' of burn care [4] is early tangential excision of eschar and autologous split-thickness skin grafting to the surgical wound areas. However, already at 20–30% of the body surface area burned the access to healthy skin for transplantation starts to be limited. Procurement of skin transplants can be repeated from the same donor site after some weeks of healing but since every procurement includes a dermal portion eventually, due to the risk of creating a full-thickness skin wound, one can usually not collect more than two or three times. Thus, culture of autologous keratinocytes is often the last resort for the most severely burned patients, and a useful tool in treating also significant burns. The skin has many crucial functions and the main goal of 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.

Culture of human keratinocytes has been in clinical use since the beginning of the 1980s [5]. Cultured keratinocytes (i.e. epithelial cells) can be used for autologous and allogeneic treatment of patients, as exemplified here.

The skin is constituted of ≈5% epidermis and 95% dermis (even though sometimes part of the subcutaneous fatty tissue is regarded as part of the skin). The dermis holds the skin's strength and pliability. In most applications today, the cultured skin is composed only of epidermal cells and keratinocytes, which restores a new epithelium (epidermis) on the patient, closes the wound and contributes 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. Thus, the quality of the healed skin, after transplantation of cultured epithelial autografts (CEA), varies equally greatly (regarding structure, function and cosmetics) depending on the residual amount of dermis.

Today, there are very few in-clinic existing therapies to restore the dermis through ordinary cell culture due to morphological appearance of the dermis with lots of extracellular matrix in a three-dimensional (3D) structure *inter alia*. In this aspect, extensive research has to be done to develop a tissue-engineered skin consisting of both dermis and epidermis useable for wound care. Meanwhile, when the option for skin substitutes is limited, the use of biological acellular allogeneic dermis (prepared from donated skin) or synthetically dermal regeneration templates can be used for reconstruction of the dermis. By applying a 3D biological degradable matrix to a surgical wound the host's cells will migrate and populate the matrix, start 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 [6].

Cultured keratinocytes can be used in two ways:

a. For permanent skin cover in an autologous manner. Autologous keratinocytes can be cultured and guided into stratified growth-rendering keratinocyte sheets which can be grafted in

the same manner as split-thickness skin grafts [7]. A novel, and alternative, way 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 [8, 9].

b. In an allogeneic situation for contribution of healing due to the release of growth factors, etc. In the allogeneic use of cultured keratinocytes on full-thickness wounds, the cells make up a temporary skin that will eventually be sloughed and be replaced by the patient's own skin (either split-thickness skin grafts or CEA). With the use of allogeneic keratinocytes on superficial dermal wounds the healing time can be shortened and since the wound is superficial dermal the possibility of spontaneous reepithelialisation is good.

22.5.2. Donor selection

In *autologous* use, donor-site selection and timing is important. To get access to as many adult progenitor cells as possible, the donor site should preferably be in a hair-bearing area. The sooner (after trauma) the skin biopsy is taken the better as the patient (and tissue) will be contaminated with microbes, thereby affecting the subsequent cell culture. However, the skin areas available for donor-site selection are determined by the extent and location of the burns.

For *allogeneic* application, donor selection must include careful examination to exclude donors with skin malignancy or other dermal conditions in addition to the general donor evaluation and testing described in Chapters 4 and 5. The transplanted allogeneic keratinocytes will be sloughed off and thus HLA-typing or ABO-blood grouping is not necessary.

22.5.3. Procurement

When procuring the skin for culture of keratinocytes it is of essential value that the site for the biopsy is located in an area with healthy skin as disconnected with the burned skin as possible. The biopsy can be either a full-thickness or split-thickness skin biopsy. A full-thickness

skin biopsy is preferred due to the amount of progenitor cell-like keratinocytes in the appendages (hair follicles, sweat glands, etc.). The yield of as many non-differentiated keratinocytes as possible is of utmost significance.

Cleanse the biopsy site properly with disinfectant ethanol (70%) without any additives, let the site air dry and carry out a second wash with sterile saline solution (9 mg/ml) before the biopsy is harvested (by e.g. scalpel). Local anaesthetics can be used *ad lib*. Immediately after harvest, place the biopsy in basal culture medium with the addition of 10% fetal calf serum (or equal substitute) and antibiotics in normal cell culture concentrations. The primary container should be sterile, closed and labelled appropriately. Transport the biopsy to the culture facility and initiate the cell isolation process as soon as possible, at the latest 24–48 h after surgical removal of the biopsy.

It is crucial to keep in mind that handling and culturing the skin biopsy/keratinocytes *in vitro* opens up the risk of contribution of (microbiological) contaminants to the cells, both from the skin itself and from culture conditions. Those hurdles can be avoided only by adequate facilities with a controlled environment (see Chapter 7), including a skilful and excellent handling technique by the staff and, if within the EU, under good manufacturing practice (GMP) conditions (see Chapter 14).

22.5.4. Processing and storage

Several procedures/protocols to isolate keratinocytes are available. It is important to use clinical-grade reagents whenever possible. This is a complex need because many of the reagents are available mainly as research-grade but the need for clinical and GMP-like grade is indisputable, and adapting the process of culturing keratinocytes in compliance with drug regulations is necessary. The two main principles of retrieving keratinocytes are:

a. Mechanical dissociation of the dermis until the biopsy mainly consists of the epidermis followed by mechanical mincing of the biopsy. The minced biopsy is repeatedly incubated in a mixture

of trypsin and ethylenediamine tetra-acetic acid (EDTA) (1:1) while gently mixing the solution for 40–60 min at 37 °C. Repeat incubation until the peak of retrieved cell amount is passed (≈4–5 cycles). If trypsin alone is used to obtain a single cell suspension, it is necessary to take into consideration that use of bovine serum albumin to stop the reaction may contradict transplantation on humans, depending on the bovine serum used.

b. Separating the epidermis from the dermis by enzymatic treatment with dispase for 12 h at 4 °C followed by mechanical release of the epidermis and mechanical mincing before further treatment with trypsin–EDTA (1:1) and gently mix the solution for 15 min at 37 °C.

Irrespective of which protocol is used, it should be properly validated before clinical application of the keratinocytes.

After isolation of keratinocytes, there are two distinctly different protocols for culturing keratinocytes; with or without the addition of serum to the culture medium.

Culturing with serum added requires seeding of the keratinocytes to a layer of feeder cells (e.g. immortalised mouse fibroblasts, 3T3). The keratinocytes will grow in polylayers, rendering the possibility to culture stratified sheets of keratinocytes to be delivered to the patient. Under serum-free conditions the keratinocytes will grow in monolayer directly on surface of culture vessel and cells can be delivered to the patient in a highly concentrated single-cell suspension to be mixed with tissue glue and sprayed on to the wound areas.

The key to success in using CEA is the priming of the recipient wound. All efforts should be taken to have a microbiologically clean wound area with a fresh granulation tissue that can provide a proper surface for the administrated keratinocytes to repopulate on in order to render a re-epithelialised area.

The first delivery of CEA for the patient is due approximately 2–3 weeks after isolation and start of culture of cells. When the first batch of keratinocytes (≤100 of sheet-grafts or the corresponding

amount of cells in a single-cell suspension) has been delivered, repeated deliveries can be done.

The amount of cells delivered and the amount of deliveries is adapted to the need for the cultured skin of the patient, the surgical procedures and the result from earlier deliveries of cells. Normally, cultured keratinocytes are used for the patient up to fourth or fifth passage of the cells. If delivery of keratinocytes needs to be paused or extended over a period of time, keratinocytes can be cryopreserved at low temperature (–150 °C or stored in nitrogen).

22.5.5. Quality controls/release criteria

The quality controls needed, besides microbiological controls (see Chapter 8), consist of continuous surveillance of keratinocyte morphology, mode of growth and expansion rate. This information decides the suitable rate of expansion for the batch. It is important to have a large cellular expansion that corresponds to the patient's need of cultured keratinocytes. However, it is equally important not to exceed the cells' capacity to proliferate. The possibility of proliferation within the keratinocytes is determined by different biological aspects, the age and health of the patient, and the biopsy site.

22.5.6. Packaging and distribution

The packing procedure is determined by the mode of delivery (i.e. sheets or suspension). Sheets are attached to an inert synthetic carrier membrane and placed one-by-one in transport liquid consisting of a basal cell culture media (e.g. Leibowitz 15). Keratinocytes delivered for spray applications are enzymatically detached from the culture vessel, washed repeatedly in basal cell culture media and finally diluted in a minimal amount of basal cell culture media (just enough to be in a solution). Further dilution is undertaken with the tissue glue in the appropriate cell concentration in the operating 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 ≈6 h) after preparation.

22.5.7. Traceability

Records to secure traceability from donor to recipient and all steps in between are under the responsibility of the tissue establishment, as described in Chapter 13.

22.5.8. Biovigilance/pharmacovigilance

As described in Chapter 14, adverse events and adverse reactions should be documented and reported. One can foresee that any event in the laboratory affecting the culture conditions, thus reducing the amount of cells or size of cell layer expected on the day of transplantation, should be considered as an adverse event.

22.6. Dendritic cells

For operators established within the EU, see Sections 20.2 and 22.2.

22.6.1. General introduction

Tumour vaccines based on dendritic cells (DC) are a new form of immunotherapy which has been tested in a large number of clinical trials internationally [10]. DC can activate tumour specific T-cells to attack and eliminate the patients' tumour. There are several subtypes of DC vaccines, but most are derived from monocytes, which are cultured in a cytokine mixture composed of granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4 and then induced into mature DC by various maturation factors.

22.6.2. Donor selection

Most DC vaccines are autologous and derived from monocytes purified from the blood circulation of patients. Donor testing includes assays for transmissible diseases, as described in Chapter 5 and, although their presence is not an exclusion criterion, it should be documented and care taken to avoid cross-contamination to other cells or

personnel. Medical evaluation of the patient should take into account the burden of collecting large amounts of leucocytes using apheresis. Depending on the method to obtain monocytes from the collected leucocytes, a pre-determined level of circulating monocytes may be relevant.

22.6.3. Procurement

As a starting material, usually apheresis-derived leucocytes are used. Apheresis should be undertaken by professionals specialising in apheresis with the precautions mentioned in this chapter for DLI collection (see Section 22.3.2).

The blood volume processed to obtain sufficient numbers of monocytes for further processing depends on the patients' peripheral blood counts, and should be calculated to avoid unnecessary apheresis time with the attendant increasing risks for serious reactions.

22.6.4. Processing and storage

Monocytes are purified from the patients' apheresis-derived leucocytes by different methods, such as counterflow centrifugal elutriation [11] or affinity column fractionation [12]. Elutriation is based on physiological (size and density) differences between monocytes and other leucocytes, whereas the presence of surface markers (e.g. CD14) is used for positive selection with the affinity column. Monocytes are then cultured for 4–5 days in the cytokines GM-CSF and IL-4. They are then induced to mature by a mixture of maturation factors (predefined according to the protocol used) in the presence of an extract from the patient's own tumour. Alternatively, mature DC could be 'loaded' with peptides derived from tumour antigens. Other attempts to obtain specificity may include insertion of specific DNA or RNA by electroporation or vectors (see Chapter 20). Aliquots of the DC vaccine are then frozen under controlled conditions or administered fresh.

22.6.5. Quality controls/release criteria

Before freezing, the mature DC are tested by flow cytometry for their expression of several cell-surface markers which are characteristic for

mature DC. These include markers such as low expression of CD14 and high expression of CD80, CD83, CD86, CD1a, HLA-DR, DC-SIGN, ILT-3 and CCR-7. Secreted markers (e.g. IL-12) can be tested by an enzyme-linked immunoassay (ELISA). It is also recommended to include a control for impurity of the cell population.

22.6.6. Packaging and distribution

Matured and aliquoted DC are frozen in cryo-tubes and stored at –150 °C in a freezer or in liquid nitrogen in a cell bank. Distribution of frozen ampoules is undertaken at low temperature, on dry ice or in liquid nitrogen.

22.6.7. Traceability

Records for the complete process from donor selection to clinical use should be kept by the responsible tissue establishment.

22.6.8. Biovigilance/pharmacovigilance

Any adverse event during collection of leucocytes, cell separation or during culturing, or adverse reactions occurring during administration, should be documented as described in Chapter 14. An example of such adverse events may be lower numbers of monocytes than expected when collected by apheresis or during cell-separation procedures. The route of administration of DC may involve adverse reactions that should be recorded and if possible avoided or at least managed according to a risk–benefit assessment.

22.7. Chondrocytes

For operators established within the EU, see Sections 20.2 and 22.2.

General introduction

Damaged articular cartilage has limited capacity for self-repair. Patients with cartilage lesions are usually related to disability and symptoms such as pain, swelling, locking and malfunction of the joint and, if these lesions are left untreated it may lead to osteoarthritis (OA). Autologous chondrocyte implantation (ACI) is used widely

for the treatment of isolated cartilage defects. The original technique (first-generation technique) is based on implantation of a suspension of *in vitro* expanded chondrocytes into the defect beneath a sealed cover of a periosteum flap. Since the technique was introduced in 1987 by Brittberg *et al.* [13] >35 000 patients have been treated worldwide.

The second-generation ACI technique involves use of a collagen membrane instead of a periosteal flap. Use of a collagen membrane simplifies the surgical procedure and reduces complications such as periosteal hypertrophy.

Further technological advances have led to the development of the third-generation technique, which involves *in vitro* expanded chondrocytes and a scaffold (briefly described in Chapter 20). After culture expansion in flasks, the cells are seeded onto a membrane or a biodegradable scaffold before implantation. The scaffold may function as a carrier for the cells or as a structure to build up the 3D environment for the cells. In most techniques, only fibrin glue is used for the fixation of the graft and, because there is no need for suturing of the periosteum/collagen membrane cover, this implantation can be done arthroscopically. After marketing authorisation, chondrocytes are manufactured and available as a product for clinical use.

22.7.1. Donor selection

The patient is examined by an arthroscopic procedure where the location, depth and size of the defect and the quality of the surrounding cartilage are evaluated. A typical patient is young with large (>2 cm^2) full-thickness chondral or osteochondral defects surrounded by healthy cartilage. At the present time, ACI is not indicated for patients with severe OA, active rheumatoid arthritis, active autoimmune connective-tissue diseases or concomitant malignancies [14].

22.7.2. Procurement

The ACI technique involves a two-stage procedure with an initial collection of a cartilage biopsy that is sent for chondrocyte culture, followed by a second-stage operation that involves cell implantation.

A full-thickness cartilage biopsy (≈200–400 mg) is harvested from a low weight-bearing area of the knee during arthroscopy. The biopsy is transferred to a sterile transport tube with biopsy medium, packed and sent to the GMP laboratory for further processing. For culture conditions with autologous serum instead of foetal calf serum, ≤200 ml of autologous blood should accompany the biopsy.

22.7.3. Processing and storage

After removal of non-cartilaginous tissue from the biopsy, the chondrocytes may be isolated from the cartilage by mechanical mincing followed by treatment with collagenase. After a digestion process in which the chondrocytes are released from the matrix, the cells are expanded in culture. For example, after 7–10 days of culture, the cells are subcultured by trypsin and seeded into new flasks or frozen down depending on the scheduled implantation date for the patient. After a total culture time of 14–21 days the correct numbers may have been reached and the chondrocytes can be prepared for application. The cells can be aseptically filled in syringes or vials. The syringes or vials are then packed for shipment to the operating theatre, where the cells are can be injected into the patient's defect under a flap of periosteum or a collagen membrane with a recommended treatment dose of ≈1–2 million cells/cm^2 defect.

During the *in vitro* expansion culture, the cells attach to the plastic, change morphology and start to dedifferentiate and gradually change their specific phenotype and become more like fibroblasts. The third-generation products include culture-expanded chondrocytes that are seeded into a scaffold that functions as a 3D support for the cells to grow and produce cartilage specific proteins. The culture period in the scaffold is usually ≈14 days, which may result in a total culture time of ≈5 weeks (including the expansion phase for the chondrocytes). The cell-loaded membranes are packed in a specific container and transported to the operating room, where the scaffold is implanted to the defect arthroscopically.

Cells may be frozen down and stored. After detachment of the chondrocytes from the culture flasks, the cells are suspended in pre-cooled freezing media supplemented with a cryoprotectant. The cell suspension is transferred to cryotubes and slow freezing (–1 °C/min) down to –80 °C is then carried out. The cryotubes are subsequently transferred to a –152 °C freezer or a nitrogen tank (gas phase) after 4–24 h. The temperature in the freezers should be monitored and should be connected to an alarm system.

22.7.4. Quality controls/release criteria

Living cells cannot be sterilised so it is very important to ensure that all handling of the product is undertaken under good aseptic conditions and that all material, media and reagents used are sterile and free of endotoxins (see Chapter 8). The first test for release is done a few days before the scheduled implantation, at the last media change, and the second test is made during assembly of the final product. The final result of a sterility test usually takes 10–14 days and the expiry time for the final product is usually 24–72 h. Therefore, it is common that the cells are already released and implanted when the final results are ready. However, a preliminary result of the sterility test can usually be given after 24 h, and it is upon this result the cells can be released. Viability is usually tested for cells in suspension using trypan blue dye. It is more difficult to test the viability of cells growing in a 3D construct. There is currently no non-destructive assay, but other release criteria specific for chondrocytes are used.

The *morphology* of the cells can be followed easily during culture using an inverted microscope. The chondrocytes should be typical for cultured chondrocytes in appearance. The personnel that carry out this subjective judgment must have good experience for this task and should have reference images of cultures for comparison.

The chondrocytes lose the phenotype of fresh chondrocytes during culture and become more like fibroblasts. The cells should do only a limited and defined number of population doublings (e.g. 4–8 population doublings). To ensure a appropriate re-differentiation of the cells,

the functional capacity of the cells after maximal expansion has to be shown, and it is important to record the *population doubling* during culture.

The *number of cells* must be stated on the product. This can either be in form of actual number of cells in the vial or syringe or the *number of cells per surface area* that have been seeded in the scaffold. Other labelling requirements are as described in Chapter 12.

To determine the possible contaminants in the product, such as synoviocytes or other type of impurities such as bone cells, the *purity* of the product is assayed. A representative batch of cells can be validated for presence of mRNA markers for chondrogenic lineage like *sox9* and lack (or low) mRNA expression of synoviocyte-specific genes. Cells are de-differentiated during culture, so more specific markers of differentiation are not tested.

Attempts to predict the *chondrogenic potential* of the cells (and thus the chondrogenic repair capacity) have been made [15] but currently no marker of clinical potency exists. Thus, functional properties of the cells and an appropriate surrogate marker are needed.

22.7.5. Packaging and distribution

The cartilage specimen is aseptically placed in a labelled sterile tube containing transport media with antibiotics that makes up for the primary packaging. The biopsy tube and blood tubes should then be placed in an outer secondary packaging that ensures the sterility and temperature and should be approved for transport of biological substances (see also Chapter 12). The biopsy should be kept cold (≈ 5–15 °C) during transport to ensure the quality of the biopsy specimen. Transport should be directly to the GMP laboratory for further processing. Processing should normally start within 48 h.

The first-generation product (cells in a suspension) is aseptically filled in syringes or vials. The third-generation product is aseptically packed in a sterile two-container system. The advantages of having primary packaging that is sterile also on the outside is that it can be taken directly to the operating table and be handled by sterile personnel.

The product should then be placed in an outer secondary packaging that ensures the sterility and the temperature and should be approved for transport of biological substances. Implantation is normally done within 48 h depending on the expiry time for the product. For accompanying information to the Organisations Responsible for Human Applications (ORHA) see Chapter 10.

22.7.6. Traceability

Records with all information from procurement to implantation should be kept by the tissue establishment. Reference samples are bio-banked.

22.7.7. Biovigilance/pharmacovigilance

Several adverse events that affect the growth and differentiation of chondrocytes may occur during the relatively long *in vitro* expansion period. In addition, if, for example, the degree of contaminating cell types unexpectedly exceeds the release criteria, this should be recorded and managed.

22.8. Hepatocytes

For operators established within the EU, see sections 20.2 and 22.2.

General introduction

Hepatocyte transplantation is an experimental treatment for patients with metabolic liver diseases, patients with acute liver failure, or patient with chronic liver failure as a temporary support to bridge to whole organ transplantation. Patients with metabolic liver diseases are characterised by deficiency of one particular enzyme or protein, giving rise to hepatic and/or extra-hepatic disease, whereas all other liver functions are unimpaired. The liver has a high redundancy in function, so selective replacement of a fraction of the liver cell mass is clearly therapeutic, and replacement of the whole liver by liver transplantation may not be required. It is assumed that replacement of 10–20% of the liver with healthy donor hepatocytes can correct a wide range of inherited metabolic liver diseases [16]. In patients with acute

and chronic liver failure, hepatocyte transplantation can give temporary liver support until the native liver has recovered or a whole organ is available for liver transplantation.

Hepatocyte transplantation has potential advantages over whole organ transplantation: the procedure provides a less surgically invasive approach resulting in less morbidity and mortality. One donor liver provides enough hepatocytes for several recipients, thereby possibly improving the ratio of donor organs to patients on the waiting list. Viable and functional hepatocytes can be isolated from marginal donor livers usually not accepted for whole organ transplantation. Also, in contrast to whole organs, cells can be cryopreserved and stored until needed.

The transplantation procedure is, in most cases, an infusion into the portal vein. The spleen and the peritoneal cavity have also been used successfully.

The generic chapters of this guide apply to hepatocyte isolation and transplantation and must be read in conjunction with this chapter. This chapter defines the additional specific requirements for liver tissue and hepatocyte isolation and transplantation.

22.8.1. Donor selection

Liver tissue for hepatocyte isolation can be procured from deceased brain-dead donors and from donors after circulatory death. Liver tissue can also be procured from living donors in the case of a patient undergoing a liver transplantation ('domino procedure') when the indication for the transplant procedure is not considered to be a contraindication for the hepatocyte recipient [17]. In theory, a healthy living donor could also donate a part of his/her liver. However, this procedure has not been done so far due to the relative risk of morbidity and mortality for the living donor in relation to the results obtained in clinical hepatocyte transplantation.

Conditions to be evaluated as part of the donor selection process are:

a. liver-originated disease of the donor which could be transferred to the recipient and cause disease (e.g. hyperoxalosis, familial amyloidotic polyneuropathy);

b. alterations to the liver vessels which could complicate perfusion and isolation of hepatocytes;

c. donor characteristics which have impact on hepatocyte quality: age of the donor, size of the liver tissue, degree of steatosis, duration of warm and cold ischemia [18–19].

22.8.2. Procurement

Liver tissue is usually collected from deceased donors by medical staff at a surgical or transplantation surgical department. Liver tissue can also be obtained from a living donor, e.g. a relative or a patient undergoing liver transplantation, when the indication for the transplant procedure is not considered to be a contraindication for the hepatocyte recipient. Staff carrying out the procurement must be dressed appropriately for the procedure so as to minimise the risk of contamination of the tissue to be removed and any hazard to themselves.

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.

22.8.3. Transportation of liver tissue to the processing establishment

Common practice is to place procured liver tissue in an appropriate organ storage solution packaged in three sterile packaging layers. This package should then be placed in another container that ensures a temperature of 2–8 °C and protects the recovered tissues during transport. Liver organ perfusion machines are evaluated for storage and transportation of liver tissue for organ transplantation and could potentially change current-day practice.

22.8.4. Processing and storage

A single cell hepatocyte suspension can be isolated from procured liver tissue. Common practice for hepatocyte isolation is a collagenase perfusion technique. First, liver tissue is divided into pieces of appropriate size. Thereafter, catheters are introduced securely into major hepatic vessels to allow for homogenous perfusion of the liver tissue. This procedure is usually done in an ice-cold water-bath to prevent rewarming of the tissue. The catheters are connected to a pump, which allows for perfusion of the liver with the three main solutions. In the first step, the liver is perfused with a crystalloid solution containing a calcium chelator that removes calcium and thus disrupts intercellular connections. Then, the liver is flushed with a crystalloid solution to remove the chelator. Next, the liver is perfused with collagenase solution to digest the extracellular matrix. During the perfusion procedure, the liver and solutions are usually kept at 37 °C to allow optimal collagenase enzyme activity. Digestion is complete after ≈15–60 min of collagenase perfusion. After mechanical disruption of the liver tissue, hepatocytes are purified by filtration and low-speed centrifugation. During this process hepatocytes are again kept at hypothermic conditions.

22.8.5. Cryopreservation, storage, and thawing of hepatocytes

Cryopreserved hepatocytes have been used successfully in clinical hepatocyte transplantation. Cryopreservation has major advantages: it improves logistics, allows for scheduled transplantations, and gives the possibility for more extensive safety and quality testing. However, freezing and thawing causes severe hepatocyte damage with decreased viability and function. Thus, further optimisation of current cryopreservation protocols is important. In current protocols, hepatocytes are kept at hypothermic temperatures after hepatocyte isolation. Cryopreservation is done as soon as possible by adding a suitable freezing solution containing a cryoprotectant (e.g. glycerol, DMSO) and using a controlled-rate freezer. After freezing, hepatocytes can be transferred to a liquid nitrogen tank and stored either in the vapour phase

(–140 °C to 150 °C) or immersed in the liquid nitrogen itself (190 °C). Hepatocytes can be stored in liquid nitrogen for ≤4 years. Longer storage periods should be validated [20].

Thawing protocols should be designed to optimise the recovery of viable hepatocytes. In current protocols, thawing is carried out rapidly to avoid formation of ice crystals. Dilution is usually done slowly to avoid osmotic imbalances.

22.8.6. Quality controls/release criteria

Hepatocytes exhibit a wide variety of functions that can be tested individually. In general, quality testing should be adjusted to the disease of the recipients aimed to be corrected (e.g. measurement of urea synthesis for recipients with urea-cycle defects, or phase-II conjugation activity for patients with Crigler–Najjar syndrome).

Cryopreserved hepatocytes have the advantage that more extensive quality testing can be carried out, which is not possible for fresh hepatocytes due to time constraints. However, current cryopreservation protocols induce severe hepatocyte damage that decrease both viability and function.

Viability and cell number should be evaluated by the trypan blue exclusion test for all hepatocyte isolations and is the single most important release criteria. In general, viability >50% is a minimum release criterion for clinical transplantation. The trypan blue exclusion test detects cell membrane damage. However, it cannot detect late apoptotic cells or metabolic function.

The following quality tests could also be considered as possible release criteria:

a. plating efficiency, ability to adhere to the extracellular matrix;

b. cytochrome P450 activities;

c. urea-cycle activity, metabolism of ammonia into urea;

d. markers of apoptosis.

None of these endpoints have specifically been demonstrated to be correlated with engraftment or proliferation of hepatocytes, *in vivo*, post-transplant, so no specific assays should be mandated. If such assays are conducted, the data from any individual assay should not be considered sufficient cause to exclude the use of the cells for a transplant. These assays will provide additional information on hepatic function that can be used in conjunction with additional data, including trypan blue exclusion, to help in the decision to use or not use cells for a transplant.

22.8.7. Packaging and distribution

Hepatocytes can either be shipped at hypothermic conditions (2–8 °C) or cryopreserved. Hepatocytes shipped at hypothermic conditions should be stored in an appropriate preservation solution. Shipping time at hypothermic conditions should be kept as short as possible as hepatocytes decrease in viability and function over time [21].

22.8.8. Traceability

Records covering the complete procedure from donor selection to recipient transplantation should be kept at the tissue establishment. 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*.

22.8.9. Biovigilance

For clinical applications of human cells, documentation of all adverse events and reactions are of particular importance as we learn and gain knowledge from them. For example, the abov-mentioned lack of *in vitro* end points that correlates with engraftment or proliferation of hepatocytes *in vivo* may only be clarified after collecting enough data and as well as adverse events during procurement and processing of hepatocytes (see Chapter 14 for management of adverse reactions).

22.9. Natural killer cells

For operators established within the EU, see sections 20.2 and 22.2.

22.9.1. General introduction

Natural killer (NK) cells were described, first in mice, and later in humans as non-B-, non-T lymphoid cells with a non-major histocompatibility complex (MHC)-restricted cytotoxic activity against transformed or virally infected cells. The 'missing-self' theory by Kärre *et al.* [22] and the identification of killer Ig-like receptors (KIR) acting as inhibitory or activating signals have contributed to the understanding and better design of clinical trials. NK cells are bone-marrow-derived from CD34 + progenitors, and migrate upon differentiation to lymphoid organs and peripheral blood. Their development and homeostasis is dependent on IL-15 and they express the adhesion molecule CD56 and lack the T-cell receptor and CD3. NK cells can be divided into at least two subpopulations according to their surface density of CD56 expression:

a. CD56 ^{bright} in a resting stage are considered to be regulatory NK cells that produce high levels of cytokines and are more proliferative, but poor mediators of NK cell cytotoxicity.

b. CD56 ^{dim} in a resting stage are potent cytotoxic cells mediating natural killer cytotoxicity as well as antibody-dependent cytotoxicity through CD16 (FcγRIII).

NK cells can be activated by several cytokines and they produce a wide variety of cytokines and chemokines (granulocyte-colony stimulating factor [G-CSF)], tumour necrosis factor [TNF]-α, TNF-β, IFN-γ, tumour growth factor [TGF]-β, macrophage inflammatory protein 1-beta, regulated on activation, normal T cell expressed and secreted [RANTES]). It is still not clear whether the *in vivo* effect of NK cells is a result of direct killing or indirectly through cytokine production, engaging other parts of the immune system. NK cell-based immunotherapies against malignancies involve either using the autologous NK cells *in vivo*, by cytokine stimulation or by adoptive transfer of autologous or allogeneic NK cells. There are many different protocols and clinical studies using NK cells against malignancies, as reviewed by Cheng *et al.*, [23] but not covered in this chapter.

22.9.2. Donor selection

Depending on the clinical protocol, autologous NK cells can be used, either by activation of the presumed NK cells *in vivo*, or by *ex vivo* selection and/or activation.

Increasing knowledge of MHC recognition (classical or non-classical) and interaction as well as the haplotypes of KIR suggests that donor selection for protocols with adoptive transfer of allogeneic NK cells could include MHC typing for HLA-C, HLA-E and possibly also HLA-F and HLA-G as well as KIR. The aim would be to select a donor whose ligands for the inhibitory signals were absent in the recipient. For adoptive transfer of *ex vivo*-activated NK cells with additional *in vivo* activation, special care should be taken to reduce the possible toxic effects of cytokines, depending on the underlying disease and dose of the cytokine(s).

22.9.3. Procurement

NK cells can be procured from peripheral blood, by apheresis carried out by experienced personnel, as described in Chapter 21 and in sections 22.3.2 and 22.6.3.

Smaller amounts of NK cells to be expanded *in vitro* are obtained from a 30–50 ml anti-coagulated venous blood sample. *In vitro* expanded NK cells can also be obtained from CD34+ stem cells from cord blood after *in vitro* differentiation and maturation with defined cytokines.

22.9.4. Processing and storage

For adoptive transfer of NK cells, an initial step of enrichment for NK cells and depletion of T-cells is essential. This is usually done by a two-step cell selection procedure. First, CD3+ T-cells are depleted and then a positive selection of CD56+ cells is carried out to obtain the NK cell population. Alternatively, if 'untouched' NK cells are preferred, negative selection by depleting all other cells (T-cells, B-cells, monocytes, granulocytes) can be carried out.

The NK cells can then be used for adoptive transfer either directly or after an initial activation step with cytokines (IL-2, IL-12, IL15, IL-18 or IFN). Additional *in vivo* activation can be undertaken by systemic administration of activating factors.

Processing should be done using reagents for human use and with predefined protocols and expectancies after each step during the cell separation process.

The final NK cell population can be frozen and stored in appropriate doses for administration *in vivo* or used as fresh NK cells.

22.9.5. Quality controls/release criteria

In addition to microbiological testing as described in Chapter 8, release criteria should include the defined level of phenotypic NK cells as established by flow cytometry, and possibly also a functional assay (cytokine production or cytotoxic assay).

22.9.6. Packaging and distribution

The processed and released NK cells are aspirated into a syringe for direct administration to the patient, with labelling containing the recipient identification and, if applicable, the dose of NK cells. Attached documentation should contain additional information to prevent mix-up if other patients or clinical trials are ongoing in the clinic (see Chapters 10 and 12).

22.9.7. Traceability

Records with all information from procurement to *in vivo* administration should be kept by the tissue establishment.

22.9.8. Biovigilance/Pharmacovigilance

Adverse events during the procurement or processing to be documented could involve less NK cell recovery than expected after a cell separation step or lack of functional activity measured *in vitro* as a quality control. Adverse reactions that should be documented could involve unexpected side-effects related to the administration of NK

cells or to the additional activating cytokines, all of which should be documented.

22.10. Pancreatic islet cells

22.10.1. General introduction

Type-1 diabetes mellitus (T1DM) is characterised by absolute and specific destruction of insulin-producing cells residing 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) [24]. DM remains a leading cause of blindness, renal failure requiring dialysis or renal transplantation, and lower limb amputation.

The ultimate goal of 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 protection from hypoglycaemia, with improved glycaemic control overall and, occasionally, complete insulin independence. It should, therefore, be available for patients with unresolved recurrent severe hypoglycaemia despite optimised specialist management.

Hence, islet transplantation may be especially beneficial in two defined subgroups of subjects with T1DM: those with intractable hypoglycaemia and those with problematic diabetic control following renal transplantation. Also, islet autotransplantation for intractable pancreatitis or other cases requiring pancreatectomy can prevent surgically induced severe DM.

22.10.2. Donor selection

Donor criteria for islet transplantation are the same as those generally applied for organ transplantation (See Part A of this Guide). All suitable deceased donor pancreases that have not been placed for vascularised whole organ transplantation may be allocated for islet transplantation according to a prioritised national waiting list. However, additional criteria for donation of tissues and cells (see Chapters 4 and 5) must also be applied.

Highest quality organ resection in addition to increased procurement rates from suitable donors have been facilitated by national organ procurement organisations throughout Europe.

22.10.3. Procurement

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.

22.10.4. Processing and storage

A 'hub-and-spoke' model may be suitable whereby islet isolation is undertaken within clinically proven islet isolation centres. Organs will be transported to the designated isolation facility. Pancreases are processed by enzymatic and mechanical dissociation and islets are collected after density gradient purification. Obtained islets are hibernated in specially designed bags (*cf.* bags used for various blood products) under stringent conditions at 20–25 °C awaiting transportation and clinical transplantation.

22.10.5. Quality controls/release criteria

In addition to the general release criteria (total number/volume of islets, sterility) the functional capacity of the isolated islets to produce insulin upon glucose stimulation should be included as a release criterion. Also, the levels of endotoxin should be determined due to their insulin-inducing effects [25]. Following confirmation of product

identity and integrity of the islet product, islets will be transplanted into the portal vein as an in-patient procedure.

22.10.6. Packaging and distribution

Isolated islets are transported in specially designed bags (*cf.* bags used for storing platelets). Hibernation temperature is usually maintained at 20–25 °C.

Islets are transported in liquid form and often in volumes >100 ml, so special notice to airline transporting regulations should be taken (see Chapters 9 and 12).

22.10.7. Traceability

Attached documentation for the clinical transplantation centre include, for example, details of the donor, organ transport/ischaemic time, pancreas quality, isolated islet number, 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*.

22.10.8. Biovigilance

Any events influencing the culture conditions are to be considered as adverse events that should be recorded and documented. Not only viability but also failure to produce the expected level of insulin upon glucose stimulation is an example of an adverse event specific to pancreatic islets.

22.11. Mesenchymal stem cells

For operators established within the EU, see Sections 20.2 and 22.2.

General introduction

Mesenchymal stem cells (MSC) are multipotent stem cells that can differentiate into various cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells). This phe-

nomenon has been documented in specific tissues and cells in living animals and their counterparts growing in tissue culture.

While the terms 'MSC' and 'bone marrow stromal cells' (BMSC) have been used interchangeably, neither term is sufficiently descriptive, as discussed below:

a. 'Mesenchyme' is embryonic connective tissue derived from the mesoderm that differentiates into haematopoietic and connective tissue, whereas MSC do not differentiate into haematopoietic cells;

b. Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSC, the term fails to convey the relatively recently discovered roles of MSC in the repair of tissue.

The term MSC, used by many laboratories today, can encompass multipotent cells derived from other non-bone marrow tissues, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp of deciduous baby teeth. The International Society for Cellular Therapy (ISCT) encourages the scientific community to adopt this uniform nomenclature in all written and oral communications when cells meet specified stem cell criteria. The specific MSC criterion, defined by the ISCT is described as:

> 'The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*.' [26].

These criteria will probably change as new knowledge unfolds. MSC are characterised morphologically by a small cell body with a few cell

processes that are long and thin. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance. The cells, which are long and thin, are dispersed widely and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils.

The immunoregulatory and regenerative properties of MSC make them an attractive tool for the development of treatments of auto-immunity, inflammation and tissue repair [27, 28]. MSC do not induce alloreactivity and generate a local immunosuppressive microenvironment by secreting cytokines, but interfere with DC and suppress lymphocyte activation and T-cell function *in vitro.* They have been shown to reverse inflammation in several experimental animal models, and clinical studies indicate that MSC are immunosuppressive also in humans becasue they reverse steroid-refractory GvHD and other inflammatory conditions [29, 30]. MSC are a heterogeneous population of cells, with functions depending on both source and *in vitro* culturing conditions. MSC are also investigated for use in tissue-engineering purposes, mainly for osteoarticular diseases and bone and cartilage regeneration. The mechanisms behind their tissue-regeneration ability as well as their immunomodulation capacity, and in particular the extent to which the two processes interact, require further elucidation. Due to the increasing interest in using MSC for human application, the safety and quality aspects to bear in mind are mentioned in this section.

22.11.1. Donor selection

Under resting conditions, MSC express HLA class I but not class II alloantigens. When cultured *in vitro* with allogeneic lymphocytes, MSC do not stimulate immune responses. Based on these findings, it has been assumed that MSC can be transfused across HLA barriers and cells from HLA-identical sibling, HLA-haploidentical relative or third party HLA-mismatched healthy volunteer donors have been used in protocols. MSC to be used for their regenerative capacity are preferably autologous.

Donors should be evaluated for their own safety and for the safety of the recipient according to the criteria described in Chapter 5.

22.11.2. Procurement

MSC can be isolated from haematopoietic tissues such as bone marrow, peripheral blood and umbilical cord blood but also from parenchymal non-haematopoietic tissues such as muscle, fat or liver. The youngest, most primitive MSC can be obtained from the umbilical cord tissue ('Wharton's jelly'). Umbilical cord-derived MSC have more primitive properties than other adult MSC obtained later in life, which might make them a useful source of MSC for clinical applications.

The two main sources of MSC presently used for human application are bone marrow- and adipose-derived MSC. The latter is one of the richest sources of MSC. When compared, there are ≈500 times more stem cells in 1 g of adipose tissue than in 1 g of aspirated bone marrow.

22.11.3. Processing and storage

MSC are rare *in vivo*, and expansion *ex vivo* is usually necessary to obtain sufficient numbers of cells required for clinical application. MSC cultures consist of a complex mixture of cell types or of cells with varying differentiation capacity and multiple differentiation stages. Their differentiation capacity *in vivo* and mode of action may strongly depend on the processing methods, conditions and duration of *in vitro* culture. Factors such as media composition (e.g. use of growth factors or serum) separation methods and cell confluence can influence the cell composition and biology. For example, it has been shown that the immunomodulation function of human MSC is enhanced when the cells are exposed to an inflammatory environment characterised by the presence of elevated local IFN-γ levels. Other studies contradict some of these findings, reflecting both the highly heterogeneous nature of MSC depending on source, and the considerable differences between isolations by the many different methods under development. It is, therefore, essential that the pre-clinical studies be done with well-defined and characterised cells, products and materials. Likewise, it is important to conduct pre-clinical studies

in the same GMP-grade facilities that will be used for clinical testing to avoid changes during the processing that can result in important differences of the final product.

MSC have the capacity to differentiate *in vitro* and *in vivo* into several mesenchymal tissues, including bone, cartilage, tendon, muscle, adipose tissue, and, possibly, bone marrow stroma.

Most commonly, expansions are done based on the cells' adherence to plastic and serial passaging. Even if MSC grow rapidly in culture, individual cells exhibit highly variable expansion potential. Furthermore, the cell yield after expansion varies with the age and condition of the donor and with the harvesting techniques. Considerable variation exists in the mode and stringency of MSC manufacture. Whether MSC are cultured on plastic dishes or other materials, for example, alters the immunophenotype of the cells considerably, and possibly their biodistribution. Other factors that influence generation of clinical products include the cell density, oxygen tension, temperature and composition of the culture medium. More recent protocols eschew foetal calf serum in favour of human plasma or human platelet lysate as additive to the culture medium. However, clinical comparative studies are lacking, and it is not known how changing the culture conditions may impact on clinical safety and efficacy.

When a sufficient number of cells have been obtained, the cells can either be infused fresh or, depending on logistics, after cryopreservation into the patient. Controversy exists whether or not frozen cells are less efficient than freshly harvested cells, and comparative clinical studies are needed to clarify this.

If frozen, the cells should be harvested, adjusted to a cell concentration suitable for the clinical application and frozen according to a validated protocol to retain viability.

Frozen MSC should be stored below –140 °C either in low-temperature freezers or in nitrogen (liquid or vapour phase).

22.11.4. Quality controls/release criteria

It is challenging to identify markers that may be predictive for the potency of a specific product with a specific indication. However, surrogate markers will be needed to address the functional properties and consistency of the cells. Further release criteria, apart from sterility (absence of bacteria, mycoplasma and fungi, see Chapter 8) may be exclusion of haematopoietic contaminating cells in the culture. Often, phenotypic release criteria include cultures predominately expressing CD73, CD90 and CD105, but the relevance of these remains to be clarified.

It is, therefore, necessary to define and validate the release criteria depending on the culture conditions used and the clinical protocol.

22.11.5. Packaging and distribution

When distributed to the clinic for administration to the patient, either fresh cells should be transferred to vials with the pre-decided dose for administration or distributed directly in a pre-labelled syringe, depending on whether the MSC are processed in the close vicinity of the clinic or not.

Frozen vials of MSC are often thawed at the bedside by diluting with isotonic saline solution at least 4× volume (to avoid toxicity by the cryoprotectant to MSC) and administered immediately to the patient. A small portion of the thawed, diluted MSC can be used to verify cell numbers and viability.

22.11.6. Traceability

Records to ensure traceability from the donation to the recipient should be kept with the tissue establishment.

22.11.7. Biovigilance/pharmacovigilance

As indicated above, MSC consist of a heterogeneous population; their phenotype and function is dependent on the source and culture conditions. Accordingly, any deviation from the expected endpoints

(according to the predefined criteria) should be considered an adverse event that should be recorded.

22.12. Limbal stem cells (ocular surface)

For operators established within the EU, see Sections 20.2 and 22.2.

22.12.1. 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 circular transitional zone between the cornea and sclera) which has the capability to continually repair and replace the surface of the cornea [31]. These cells are known as limbal stem cells (LSC). LSC deficiency, a term which covers acquired pathological deficiencies such as physical or 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 [32]. These anomalies are very difficult to treat because of the significant impairment of the patient's vision which, in most cases, progresses to total blindness. LSC have the characteristics of undifferentiated stem cells and are capable of undergoing proliferation and differentiation into the corneal epithelium. They may also be genetically modulated for therapeutic purposes [33].

Currently, approaches to the treatment of many ocular surface diseases focus mainly on two aims: (a) repair/regeneration, by obtaining epithelial cells from the corneal surface (cornea and conjunctiva) or other suitable non-ocular tissues or (b) ex vivo expansion of LSC and *in vitro* culture for subsequent bio-substitution. Achieving effective control of the underlying inflammatory process and tissue rejection is the key objective [32–33].

22.12.2. 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 LSC 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 sometimes used as a scaffold for growing tissue explant and cell cultures respectively, and then used for transplants (see Chapter 20). Bilateral LSC deficiency, on the other hand, is a devastating pathological condition affecting both eyes; in this case, autologous limbal tissues or cells cannot be sourced from the same patient because both eyes are affected. Alternative sources of transplantable tissue include the culture of epithelial cells lining the autologous oral mucosa. Allogeneic sources of transplantable tissue, from diseased donors, may be an option for restoring the function of the ocular surface, although procedures with autologous healthy tissue are always preferable to those using heterologous sources [34, 35].

22.12.3. Procurement

Extraction as well as *ex vivo* expansion/*in vitro* cultures must be carried out in strict laboratory conditions that comply with GMP requirements. The explants (autologous or allogeneic) are obtained by a biopsy of healthy ocular tissue from the limbal region and maintained in sterile culture medium until processed. The most widely applied technique, with minimal manipulation of tissue, is the explant culture system, in which the specimen obtained by biopsy is directly placed in the centre of the amniotic membrane.

Epithelial cells (LSC) grow out from the biopsied tissue and proliferate to form a multilayered epithelial construct.

An alternative approach, with additional manipulation of the tissue, is to obtain single epithelial cell suspensions by enzymatic isolation (a one- or two-step enzymatic method) from limbal specimens.

22.12.4. Processing tissue and storage

Epithelial culture medium is carefully added to the limbal biopsy that is gently pressed down to facilitate attachment to the plated amniotic membrane. Early explant outgrowth is observed at 2–3 days but full coverage of the amniotic membrane usually requires *in vitro* expansion of the limbal tissue for *ex vivo* expansion 15–21 days after the initial limbal biopsy.

The explant culture system does not allow storage procedures [32].

For *in vitro* culture of cells, several protocols use xenobiotics or allogeneic products and undefined components in the culture medium, such as bovine serum or animal-derived supplemental additives. LSC growth in culture may need additional feeder-layer support, and is often carried out with foetal murine fibroblast cells previously irradiated or inhibited by mitomycin-C treatment. Currently, efforts are being made to avoid or replace the use of xeno-/allo-derived products in cell culture protocols [32–34]. In the enzymatic method, the biopsy specimen is first treated with dispase solution to separate the epithelial sheet from the rest of the specimen. Thereafter, the epithelial sheet is immersed in a solution of trypsin to obtain a homogeneous suspension of LSC. Cells are then co-cultured with a feeder-layer of clinical-grade certified lethally irradiated 3T3 murine fibroblasts. Once the culture is established (10–15 days), the cells are harvested with trypsin solution and stored in a cryoprotective solution in a liquid nitrogen (–196 °C) tank. A portion of the culture is used to test its quality using microbiological assays, immunocytochemistry for CK3, CK19, p63, clonogenic assays, and checking for the presence of murine 3T3 cells. Once the quality of the culture obtained is confirmed, cells are seeded on human fibrin gel and maintained under cell culture conditions. At present, this technique is known as cultivated limbal epithelial transplantation (CLET) [34, 35].

Epithelial cells from the oral mucosa may be obtained by a similar method and the final approach is known as cultivated oral mucosal epithelial transplantation (COMET) [36].

22.12.5. Quality controls and release criteria

All handling of the products and supplement components requires GMP, including facilities (clean rooms and laminar flow hoods), and that all the material, media and reagents used are sterile and endotoxin-free (see Chapter 7). Microbiological tests that simulate normal processing conditions by replacing the cells with culture media are essential. Three simulations of all the critical stages of the process should be done before the implementation process [37].

Conventional microbiological (bacteria and fungi) assays should be undertaken at baseline, during the process, and in the final product before clinical use. Specific tests for the presence of *Mycoplasma* spp. are conducted after handling the final product. Morphological control of culture cells and bioengineered products can be controlled with the use of a phase contrast microscope. Basic cell culture techniques can be applied, such as cell viability tests with vital dyes and techniques for cell counting using an automated cytometer or cell counting chambers.

In the explant culture system the final product requires less handling, and adequate microbiological control of the final product (LSC on amniotic membrane) is needed to obtain approval for clinical use. Further release parameters are to be established during the pharmaceutical and clinical development of the cell based product.

22.12.6. Packaging and distribution

The purpose of packaging and labelling operations is to protect the product and to provide identification and information for the user. Packaging and labelling are key elements in the Quality Management System to ensure that appropriate standards are maintained during a product's lifecycle [37].

The final engineered cell product should be conditioned in a suitable sterile container immersed in fresh complete cell culture medium and maintained at temperatures validated to ensure the viability of the cells until arrival at the transplant centre (see Chapter 12). A maximum period should be set for delivery and it is recommended that the product is implanted on the same day as its release for clinical use, unless longer storage is adequately justified. As described in Chapter 10, the essential information about usage of the cells should be included in accompanying documentation to the ORHA.

22.12.7. Traceability

Special consideration should be given to the reagents that come into contact with tissues and cells and may be left as residues when cells are applied in the recipient. The following links in the process are particularly important: between the donor and donation at the tissue centre, between donation and the final cell product at the manufacturing site, and between the final bioengineered product and the recipient at the transplant centre.

22.12.8. Biovigilance/pharmacovigilance

As described in Chapter 14, deviations from the standard SOP, from donation to clinical application, should be recorded and documented as well as adverse reactions after application.

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Chapter 23

Adipose tissue

Autologous adipose tissue from liposuction is being used increasingly in plastic surgery for reconstructive procedures. Some of the implanted tissue is resorbed, so surgeons treating large defects frequently apply a staged approach. This approach can be facilitated by storing all or part of the tissue collected from the initial liposuction and implanting it during subsequent interventions. 'Fat banking' eliminates the need for repeated liposuction and, thereby, reduces cost and the risk of morbidity. One of the most common indications for this therapy is reconstruction after tissue removal in patients with breast cancer. The indications for this procedure could also include lipodystrophy due to acquired immune deficiency syndrome, so positive results in the serology test can be accepted for autologous use. Risk assessment on the conditions of procurement, processing facilities and storage should be conducted and appropriate mitigating actions taken to prevent cross-contamination.

The generic chapters of this Guide are relevant to the banking of fat tissue as they are for any other type of tissue used in clinical applications. The criteria for donor selection to be applied are those for autologous donation; the patient must be provided with sufficient information regarding the process (including the planned storage period) and should sign an informed consent form.

This chapter does not cover adipose tissue as a source of stem cells.

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 addition of an 'antibiotic cocktail' to the lipoaspirate. All bags or containers used in the collection, processing and final packaging (frozen or thawed tissue) must be labelled appropriately.

Tissue processing includes centrifugation, use of cryoprotective agents such as glycerol, trehalose or dimethyl sulfoxide and controlled-rate cooling (≈1 °C/min) and storage below –130 °C (vapour- or liquid-phase nitrogen) to preserve maximum viability of adipocytes. The thawing protocol must remove the cryoprotectant as well the amount of free lipids and debris associated with loss of adipocytes. Quality control must include microbiological testing of each batch; histology and cell-viability controls are highly recommended.

Any adverse reaction or events occurring during processing, thawing or re-injection of tissue should be notified as described in Chapter 14.

In recognition of the growing importance of this type of tissue banking, and its potential extension to an allogeneic setting, a full, tissue-specific chapter will be developed for the third edition of this Guide.

Chapter 24

Other substances of human origin for clinical application

24.1. Introduction

This chapter introduces several other substances obtained from humans for autologous or allogeneic use. In some cases, their therapeutic effectiveness is questionable; in others it is very clear. In general, their regulatory classification is challenging because they do not fit clearly into one group of therapies or another, whether the criterion applied is method of application, mode of action, or complexity of processing. Hence, a wide variety of approaches to regulation exists in Europe and, in some cases, these substances are banked outside of any regulatory framework. 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.

Regardless of their regulatory status, ensuring safety and quality requires a similar approach as that applied for the tissues and cells discussed in the other chapters of this Guide. Thus, guidance for the selection and testing of donors as well as quality management

and traceability described in the generic section of this Guide can be applied usefully to the banking of these substances to achieve an appropriate framework for providing safe and effective services to patients.

24.2. Breast milk

Banks for human milk that collect, store, process, screen and distribute breast milk from donors have existed in Europe since in 1909 (in Vienna, Austria) [1]. There are ≥186 milk banks operating throughout Europe [2]. Many countries have national guidelines provided by a professional society or developed by the Health Authority. An example is *Donor breast milk banks – the operation of donor milk bank services* published by the National Institute for Health and Clinical Excellence in the UK in 2010 [3].

Donor breast milk is used mostly to feed preterm babies in neonatal intensive care units when the mother's own breast milk is not available. It offers an alternative to nutrient-dense 'preterm formulas'. It may also be used for infants undergoing surgery or those with intrauterine growth restriction or cardiac problems. Common agreement on the clinical criteria for the use of donor milk is lacking. Although the benefit is nutritional, a recent comprehensive review of the literature confirmed that feeding with donated breast milk has a protective effect against necrotising enterocolitis (NEC) in premature infants [4]. NEC may be caused by the detrimental effect of proteins from cow's milk on the developing human intestine. This protective effect is achieved through supply of immunoprotective factors to the immature mucosa (though the absence of harmful antigens may also be a contributing factor) [5]. Feeding of donor breast milk may also protect against bronchopulmonary dysplasia, and may have beneficial effects on cardiovascular risk factors measured during adolescence, though the evidence in both cases is not conclusive. Recently, a research team reported the potential use of human donor milk as a source of stem cells; this development could have a significant impact on milk-banking services and their regulation [6]. However, no evidence has been provided that these stem cells could be of clinical use.

The risks associated with feeding with donor breast milk are similar to those in the food industry (i.e. contamination or cross-contamination with bacteria or fungi with subsequent recipient infection) but are also equivalent to those associated with transfusion and transplantation (i.e. transmission of viruses and other infective agents).

Donated milk should be provided by well-organised banks that select and test donors in a manner that is equivalent to that outlined in Chapters 4 and 5 of this Guide. In addition to the exclusion criteria described in those chapters, many guidelines exclude smokers of tobacco or cannabis, and women that consume more than a defined amount of alcohol or caffeine each day. Testing should be done before the first milk collection and a schedule for repeat testing should be defined. Italian National Guidelines (available in English) provide a very useful *rationale* for the testing and selection requirements of donated milk [7].

Banks should have in place comprehensive quality management in accordance with that detailed in Chapter 3. Donated milk should be processed under hygienic conditions (a sterile environment is not necessary). It should be sterilised before use. Standard practice is to pasteurise at 62.5 °C for 30 min (Holder method) in a human milk pasteuriser followed by cooling to ≤4 °C and then freezing. Milk can be pasteurised as single donations or as pools.

Before pasteurisation, a sample from each batch of pooled donor milk should be tested for microbial contamination. A policy should be in place for discarding milk pre-pasteurisation. According to UK National Institute of Health and Clinical Excellence (NICE) Guidelines, milk is discarded before pasteurisation if:

a. total viable bacteria count $>10^5$ colony-forming units (CFU)/ml or

b. *Enterobacteriaceae* $>10^4$ CFU/ml or

c. *Staphylococcus aureus* $>10^4$ CFU/ml.

Other countries have similar (but slightly) different approaches. These different approaches are reviewed in the Italian National Guidelines, which state that a total bacterial count $>10^5$ is an indicator of

poor-quality milk. Based on this criterion and on the theoretical concern that heavily contaminated milk with specific bacteria (e.g. *Staphylococcus aureus, Escherichia coli*) may contain enterotoxins and thermostable enzymes even after pasteurisation, the Italian expert panel selected 10^5 CFU/ml for total bacterial count, and 10^4 CFU/ml for *Enterobacteraceae* and *Staphylococcus aureus* as threshold values. These pre-pasteurisation values are in accordance with the NICE Guidelines and similar to French legislation.

After pasteurisation, frozen donor milk should be stored for ≤6 months after the date of donation.

There should be a planned schedule for microbiological testing of pasteurised milk. The testing schedule should be based on the volume and throughput of milk. NICE Guidelines recommend:

a. either at least once a month or every 10 cycles (depending on which comes first); and

b. on an *ad hoc* basis if any new processes, equipment or staff are introduced, or if there are concerns about any part of the process.

For certain medical conditions, non-pasteurised milk may be given to premature babies [8].

In the European Union (EU), human breast milk may fall within different national legal frameworks for which appropriate quality and safety requirements need to be applied (e.g. food, tissues and cells).

24.3. Faecal microbiota

Faecal microbiota transplantation (FMT) was first reported in 1958 by Eiseman *et al.* [9]. Since then, promising reports of FMT have suggested a clinical cure rate of ≈90% for recurrent *Clostridium difficile* infection refractory to antibiotic therapy. The transplant can restore the diversity of gut microflora, which may confer protection against toxigenic *Clostridium difficile* [10, 11].

In a systematic review and meta-analysis conducted recently, 11 studies concluded that FMT is a promising intervention for serious recurrent

Clostridium difficile infection after conventional treatments have been exhausted [12] and a small randomised controlled trial published in 2013 confirmed this finding [13]. The effectiveness of FMT for treatment of ulcerative colitis has also been reported [14].

Traditionally, donors have been close relatives or spouses. However, there would be considerable benefits in having unrelated pre-screened donors to allow more rapid transplantation if it is needed. There may be benefits in using donors from different households, where the gut microbiome would be different and would provide enhanced diversity in the patient.

Transplantation carries risks of disease transmission. Donors should be selected and tested carefully just as for other types of transplantation of tissues or cells, though testing for infectious markers might not be required for a donor who is in a sexually intimate relationship with the recipient. The optimal frequency of testing is not known, though a schedule should be defined if multiple transplants are to be carried out. Along with the usual donor-exclusion criteria described in Chapter 4 of this Guide, some specific exclusion criteria have also been proposed:

a. history of inflammatory bowel disease, idiopathic chronic constipation, or chronic diarrhoea;

b. history of gastrointestinal malignancy or known polyposis;

c. antibiotic treatment within the preceding 3 months;

d. use of major immunosuppressive medications (e.g. calcineurin inhibitors, exogenous glucocorticoids, biological agents);

e. systemic anti-neoplastic agents;

f. recent ingestion of a potential allergen (e.g. nuts) whereby the recipient has a known allergy to this agent.

Donor faeces are obtained and diluted with water, saline or another liquid (e.g. milk, yogurt) and filtered to remove large particles. The resulting suspension is introduced into the recipient's gut *via* a nasogastric tube, nasoduodenal tube, rectal enema or biopsy channel of a

colonoscope. Recipients may receive a bowel lavage before transplantation to reduce the *Clostridium difficile* load in the intestines.

Donated faces should be tested as follows:

a. *Clostridium difficile* toxin B by polymerase chain reaction or evaluation for toxins A and B by enzyme immunoassay (EIA);

b. routine bacterial culture for enteric pathogens;

c. faecal *Giardia* antigen;

d. faecal *Cryptosporidium* antigen.

The NICE has also published guidance on interventional procedures on FMT [1].

In the EU, FMT may fall within different national legal frameworks for which appropriate quality and safety requirements need to be applied (e.g. medicinal products, tissues and cells).

24.4. Teeth and dental pulp

Several commercial companies have begun to offer services of banking of tooth pulp or dental pulp. They suggest that the tissue, although of no therapeutic value now, could be useful as a source of mesenchymal stem cells in the future. Stem cells have been identified in teeth [15–19] but evidence that they could be of clinical use in the future is hypothetical. If tooth pulp or dental pulp might be used for human applications in the future, all of the requirements of the generic section of this Guide should be respected.

24.5. Platelet-rich plasma and platelet-rich fibrin

Platelet-rich plasma (PRP is an autologous platelet concentrate in a small volume of plasma. PRP is used in several surgical procedures together with musculoskeletal tissues. Platelets release seven main factors responsible for stimulation of healing process. There are primarily three isomers of platelet-derived growth factor (αα, ββ, αβ), transforming growth factor (TGF)-β1, TGF-β2, endothelial growth factor (EGF) and vascular endothelial growth factor (VEGF). PRP also

contains the proteins responsible for cell adhesion: fibrin, fibronectin, and vitronectin [20].

In contrast, platelet-rich fibrin (PRF) does not produce the very high platelet concentrations seen in PRP. The *rationale* for PRF use is formation of a three-dimensional cross-linked fibrin matrix that is essential to the platelet plug because it serves as a binding site for platelets and growth factors (GF). This 'scaffolding' helps localise the GF, essentially increasing the local concentration at the desired location to guide tissue regeneration [21].

In the EU, PRP may fall within different national legal frameworks for which appropriate quality and safety requirements need to be applied (e.g. medicinal products, blood, tissues and cells).

24.6. Serum eye drops

Serum eye drops have a potential advantage over traditional therapies for dry eye because human serum is not only lacrimal substitute, but also contain growth factors and other biochemical components mimicking natural tears more closely. This is the reason why serum eye drops become popular second-line therapy in dry eye treatment.

Serum eye drops are usually prepared for autologous use from the donor's serum. In the case of repeated collection of blood to prepare serum eye drops, special attention should be paid to avoid the development of anaemia. Each time before blood is collected, donors must be tested at least for hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and human immunodeficiency virus (HIV). Contraindications for eye drops production are elevated bilirubin or protein, active viral or fungal infection, and certain medications that may injure the cornea.

The patient must be given appropriate information about the blood collection process, eye drop preparation and written instructions for storage and handling of the eye drops at home.

There must be written protocols for all procedures related to blood collection and eye drops production. All measures should be taken to

minimise the risk of microbiological contamination, including disinfection of the phlebotomy site using methods accepted for collection of blood for transfusion. Blood should be collected in a sterile container/blood bag, without anticoagulant. The collected blood volume depends on local procedure but cannot be more than for regular blood donors. Eye drop preparation should be carried out using aseptic technique. It is strongly recommended to use a closed system for aliquoting. Microbiological control for each batch is mandatory. The volume of one aliquot shall be adjusted for daily dose to minimise microbiological contamination in the patient's home. All bags that are used in the collection, processing, and/or aliquots of final packaging must be properly labelled. Eye drops must be stored frozen and transported in an appropriate container, to maintain the required temperature. The shelf-life for frozen storage is 3–6 months.

It is strongly recommended that the ophthalmologist monitor the patient's progress in a standard way because scientific data on the benefits of using serum eye drops is still under research. Any adverse reaction or events occurred during eye drops production or usage should be notified.

In the EU, serum eye drops may fall within different national legal frameworks for which appropriate quality and safety requirements need to be applied (e.g. medicinal products, blood, tissues and cells).

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Chapter 25

Assisted reproductive technology

25.1. Introduction

Assisted reproductive technology (ART) refers to medical procedures used to achieve pregnancy involving the collection, processing and/or storage of at least one of the following reproductive tissues and cells: oocytes, ovarian tissue, sperm, testicular tissue, and embryos. ART is carried out using freshly collected and/or cryopreserved gametes, zygotes or embryos originated from the couple being treated ('partner donation') and also from gamete donors ('non-partner donation'). These contexts are, in general, addressed separately due to the different risks involved. In this chapter, ovarian stimulation or any other clinical procedure that does not involve gamete collection will not be addressed.

ART comprises methods such as:

a. Artificial insemination, mostly intrauterine insemination (IUI). Sperm is provided by the partner or from a donor, processed and placed in the uterus at the estimated time of ovulation, thereby leading to *in vivo* fertilisation.

b. *In vitro* fertilisation (IVF), either conventional (whereby collected and prepared sperm and oocytes are placed in contact in

culture) or intra-cytoplasmic sperm injection (ICSI; whereby a single spermatozoon is injected into an oocyte). IVF and ICSI involve collection and processing of gametes, fertilisation, culture and, eventually, transfer, cryopreservation and storage of embryos.

c. Cryopreservation of gametes, embryos, and/or germinal tissue.

Pre-implantation genetic diagnosis (PGD)/pre-implantation genetic screening (PGS) are procedures linked to IVF that use genetic identification methods to diagnose or screen *in vitro* embryos for genetic abnormalities to select embryos before transfer. In some countries, PGD and/or PGS are not allowed or allowed only in specific circumstances according to national legislation.

ART is carried out in ART centres (tissue establishments dealing with assisted reproduction bringing together a clinical unit and a laboratory in a multidisciplinary team) to treat patients with fertility problems. In various indications (e.g. cancer, some chronic diseases) for which treatment may be potentially harmful for fertility, long storage of cryopreserved reproductive tissues and cells may be proposed to children, adolescents and young male and female adults. This approach, called 'fertility preservation', will be addressed in Chapter 26.

ART can also be proposed to couples at risk of transmission of a serious communicable disease (e.g. human immunodeficiency virus [HIV] or hepatitis B and C viruses [HBV and HCV, respectively]) infection) to the partner and to the child. In some countries, ART can be undertaken in unmarried women or homosexual female couples. In a few countries in Europe, under stringent conditions, surrogate motherhood is allowed for women without a uterus or with a non-functional uterus, or for male homosexual couples. Through insemination or embryo transfer, the surrogate mother carries and gives birth to a child for the intended 'social parents'.

ART has been developed in most countries in the world. Each year, the European Society of Human Reproduction and Embryology (ESHRE) publish a report of activity in European countries based on voluntary declarations. ESHRE data from 2010 reported 550 296 ART

treatment cycles, including IVF, ICSI, frozen embryo transfers, ART with oocyte donation and PGD/PGS, in 31 countries. In addition, 212,636 IUI, including partner and non-partner IUI, were declared by 22 countries. In countries in which ART is well established, 2–4% of children born per year are conceived using ART.

ART involves ethically sensitive medical activities that lead to the birth of a child. As such, the described procedures are intended to achieve efficient results in terms of delivery rates and also address the safety of patients, donors and children born after ART. For partner donation, priority is given to use the reproductive cells from the partner even if the sample exhibits poor quality (e.g. ART between partners with a partner infected by HIV; ICSI for patients with decreased sperm quality; ART between partners with a well-known risk of transmission of a genetic disease).

This Guide aims to provide guidelines which can help to conceive healthy singletons carried to term through ART.

The following generic chapters of this Guide (Part A) all apply to ART 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; only for non-partner donors);

d. Distribution and import/export (Chapter 9);

e. Organisations responsible for human application (Chapter 10);

f. Computerised systems (Chapter 11);

g. Packaging and labelling (Chapter 12); only for non-partner donors;

h. Traceability (Chapter 13);

i. Biovigilance (Chapter 14).

This chapter defines the additional specific requirements for ART.

25.2. Consent

As mentioned above, ART can be undertaken with partner- or non-partner-donated gametes (partner donation or non-partner donation). Chapter 3 clearly describes giving consent for donation by living donors, and this situation applies for gamete donors in the case of non-partner donation. For partner donation, fully informed written consent is also mandatory and additional aspects that should be addressed concerning these specific consent forms are described in this section.

In partner donation, the couple starts an ART treatment to fulfil their wish to have a child together. Couples should receive written and verbal information concerning the national legislation about ART and the possibility of reimbursement (if applicable). In addition, the couple must be informed about the possibilities of success ('live birth rate') based on their individual medical history. The degree of invasiveness of the treatment and medical risks (e.g. risk during ovarian stimulation, risk of multiple pregnancies, incidence of abnormality of children conceived through ART) should be addressed. Psychological counselling should also be offered to the couple. Use of an information sheet or brochure to aid the couple through an ART treatment is advisable.

ART treatment normally comprises a series of individual treatments, so consent forms for each ART treatment should be signed or be valid for consecutive ART treatments until the treatment is successful, until a predefined date, or until relevant circumstances change.

If the ART treatment is undertaken with cryopreserved gametes or embryos, consent for thawing should be given for each ART 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 the remaining (supernumerary) embryos or gametes should be cryopreserved or not. The couple giving consent to cryopreservation should receive information regarding the different options on the fate of gametes or super-

numerary embryos. Embryos must not be created for purposes other than for reproduction. According to national legislations, the destiny of gametes or supernumerary embryos could be donation to other couples, donation for scientific research or destruction if there are changes in the parental project. The consent form could also specify the duration of storage.

There should be specific consent whenever additional methods beyond IVF/ICSI and cryopreservation are used. A very specific case in ART is the possibility to consent for use of donated gametes after the donor has died ('posthumous donation'). In some countries, fulfilment of the child wish of the couple using the remaining gametes or embryos after one of the partners has died is allowed. This factor needs to be specified in the consent form that both partners sign at the start of the treatment.

The couple (or individual) should declare in writing their consent to undergo an ART treatment. The couple (or person) should declare in writing to be fully informed about the:

a. possibility of adoption, foster care and alternative options for the couple to have a child;

b. national legislation about ART and its implications for those who have access to assisted reproduction;

c. possibility of withdrawal of consent to treatment;

d. possibility of the physician not to proceed with the entire treatment (or some of its parts) for medical or deontological reasons;

e. route of the ART cycle at each stage of its implementation;

f. possible ethical issues regarding ART;

g. possible psychological effects resulting from ART application;

h. chances of success, the degree of invasiveness and the possible risks of the ART treatment (including multiple pregnancies). Specifically in IVF, special reference must be made to ovarian hyperstimulation syndrome (OHSS) and the risks linked to

oocyte retrieval (e.g. bleeding, infection or perforation of the bladder or bowel);

i. possible risks to the offspring resulting from ART, particularly in multiple pregnancies, and the limited follow-up data on the long-term health of those born from ART;

j. testing for genetic and infectious disease and evaluation carried out in gamete donors in non-partner donation.

k. possibility of cryopreservation and storage of gametes and supernumerary embryos, and the options for future use according to national legislation;

l. total cost of the ART procedure.

A female partner who enters IVF treatment could decide to donate several oocytes. This procedure is called 'oocyte sharing' and implies that she should be considered as a donor in non-partner donation. In this case, the patient should be considered a partner and non-partner donor simultaneously. All possible measures should be taken to avoid that women feel pressured to donate oocytes in order to be able to receive the treatment needed for them to have a child.

25.3. Donor evaluation

25.3.1. Evaluation of partner donors

25.3.1.1. *Interview*

Couples who experience problems in conceiving should be seen together because infertility constitutes a medical problem for the couple. Both partners need to be aware of the decisions surrounding their investigation so that they can support each other. Counselling before, during and after treatment is widely practiced and recommended. This is because fertility problems, their investigation and treatment can cause psychological stress.

25.3.1.2. *Taking of medical history and physical examination*

Full medical, surgical, sexual, contraceptive, genetic, family and pregnancy history should be taken from both partners. This information will help to determine the cause of the couple's infertility, the appropriate investigations to undertake, and help to determine the best course of treatment. A history of coital frequency, dyspareunia and any sexual dysfunction should also be elicited from the couple. Both partners should undergo a physical examination.

25.3.1.3. *Female investigations*

a. Assessment of ovulation

A complete menstrual history should be taken, including age at menarche, cycle length, frequency, and the presence/severity of dysmenorrhoea. Ovulatory dysfunction can be due to hypothalamic, pituitary or ovarian dysfunction. Ovulation can be confirmed retrospectively by measurement of serum levels of progesterone in the mid-luteal phase of the cycle. Women with irregular menstrual cycles should also have serum levels of gonadotropins measured (i.e. follicle-stimulating hormone (FSH) and luteinising hormone (LH) to assess hypothalamic pituitary function).

b. Assessment of ovarian reserve

Measurement of ovarian reserve may include biochemical tests and ultrasound imaging of the ovaries, especially antral follicle count (AFC). Biochemical tests may include basal measurements of FSH, inhibin B, and anti-Müllerian hormone (AMH). No single test has 100% sensitivity and specificity, so biochemical and imaging measurements are combined in an effort to improve their validity. However, there is insufficient evidence to recommend that the result of any single ovarian-reserve test should be the sole criterion for use in ART.

c. Assessment of tubal patency

Hysterosalpingo-contrast sonography (HyCoSy) may be used in an outpatient setting to assess tubal patency. Hysterosalpingography (HSG) may also be done as an outpatient procedure to assess tubal

patency, uterine anatomy and assess the site of tubal blockage. Laparoscopy plus dye test is the 'gold standard' method used to assess and confirm the site, aetiology and degree of tubal blockage. Laparoscopy also allows the diagnosis of other conditions (e.g. endometriosis). Consideration should be given to testing of *Chlamydia trachomatis* in women with tubal damage.

d. Assessment of uterine abnormalities

Hysteroscopy may be considered if uterine abnormalities such as submucous fibroids, polyps, adhesions or septae are suspected on ultrasound or HSG. A concomitant laparoscopy may be indicated in certain circumstances.

e. Serology

Testing for immunity to rubella should be carried out before treatment. Vaccination should be offered to seronegative women before they commence an ART cycle.

25.3.1.4. *Male investigations*

a. Semen analyses

Semen analyses should be carried out before ART. Reference values are described in the *WHO Laboratory manual for the examination and processing of human semen* [1]. To confirm any abnormality detected, analyses of a second semen sample should be done. Results of semen analyses are used to determine the most appropriate ART method.

b. Investigations to determine cause of abnormal semen

Severe oligozoospermia (<5 million sperm/ml) and azoospermia may be caused by testicular failure. Testicular failure may be due to trauma, infection, varicocele, gonadotropin deficiency, tumours e.g. testicular cancer, Hodgkin's lymphoma, leukaemia or associated gonadotoxic therapies or to genetic abnormalities such as Klinefelter syndrome or Y-chromosome deletions. Men with azoospermia and severe oligozoospermia should be screened for the above-named genetic abnormalities. If a chromosomal abnormality is detected, appropriate genetic counselling should be offered. Cystic fibrosis or

renal-tract abnormalities should be screened for in the presence of obstructive azoospermia. Besides genetic testing, men with testicular failure should have hormonal testing and a scrotal ultrasound.

25.3.1.5. *Inclusion/exclusion criteria for treatment*

A full medical evaluation will determine if a couple is suitable for ART treatment. The risk–benefit analysis should be estimated on an individual basis.

The maximum recommended number of cycles for partners undergoing ART treatment may vary from clinic to clinic and country to country. The number of repeat cycles should be based on the individually estimated probability of a live birth.

25.3.2. 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 (e.g. infectious disease, genetic disease) can be excluded. Ensuring that the donation process does not cause harm to the health of the donor is also important.

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 and rhesus typing;

g. genetic testing as indicated by family history and prevalence of carrier status in specific populations. Karyotype testing may also be recommended;

h. semen analyses for sperm donors. Freezing-thawing test may also be recommended;

i. assessment of ovulation and ovarian reserve (including endocrine work-up) in oocyte donors;

j. informed consent before any procedure.

25.3.2.1. *Exclusion criteria for oocyte donors*

a. Age <18 years or >36 years;

b. Positive results in tests for genetic disease and/or infectious disease;

c. Any risk factor to her own health;

d. Unsuitability for donation based on interview.

25.3.2.2. *Exclusion criteria for sperm donors*

a. Age <18 years and >45 years;

b. Positive results in tests for genetic disease and/or infectious disease;

c. Unsuitability for donation based on interview;

d. Unsuitability for donation based on interview.

In cases in which embryos are donated, partners that have generated them should be considered non-partner donors and comply with general criteria for non-partner donation.

25.3.2.3. *Welfare of oocyte donors*

The welfare of oocyte donors is very important. The risk of OHSS should be minimised. The number of times an oocyte donor may donate should be determined by several factors: number of children and/or families achieved with this donor's gametes; medical and psychological risks to the donor; legislation in the country of donation; where the donated gametes are used. ART centres should be encouraged to accept female donors after they have achieved a successful pregnancy and consider the number of times a woman may donate by using appropriate Registries.

25.4. Testing

The purpose of testing gamete donors is to prevent transmission of infectious and genetic diseases from the donor to the recipient and offspring and to protect the staff while handling the patients and their gametes.

Testing of gamete donors will be discussed differently for each type of donation:

a. partner donation

b. non-partner donation.

In the donation of reproductive cells between partners who have an intimate physical relationship (partner donation), less strict biological testing is justifiable because the risk to the recipient is considered lower than that from donations by non-partner donors.

25.4.1. 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, each tissue establishment, upon analyses of risk, can also carry out additional tests:

d. Syphilis (a treponemal-specific test or non-specific treponemal test can be used);

e. Testing for human T-lymphotropic virus (HTLV)-I 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.

In certain circumstances, additional testing may be required depending on the donor's history of travel and exposure as well as the characteristics of the tissue or cells donated (e.g. RhD (D antigen), diagnostic tests for malaria, antibodies to cytomegalovirus (CMV),

chlamydia, antibody to *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 by the same donor, additional blood samples must be obtained according to national legislation, but ≤24 months from the previous sampling.

Positive results will not prevent partner donation. Robust procedures should be in place to prevent the risk of contamination to personnel and cross-contamination. If results for tests of HIV-1 and -2, HBV or HCV are positive or unavailable, or if the donor is known to be a source of infection risk, a system of separate handling and storage must be devised.

In the case of sperm processed for IUI and not to be stored, 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.

25.4.2. 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.

All serum samples must be obtained at the time of donation.

Testing for HTLV-I antibodies must be done in donors living in or originating from high-prevalence areas or with sexual partners orig-

inating from those areas or where the donor's parents originate from those areas.

In certain circumstances, additional testing may be required depending on the donor's history of travel and exposure and the characteristics of the tissue or cells donated (e.g. RhD (D antigen), diagnostic tests for malaria, antibodies to CMV, antibody to *Trypanosoma cruzi*).

Sperm donations must be quarantined for ≥180 days after the last collection, after which repeat testing is required. If the initial blood sample is tested by NAT for HIV, HBV and HCV, testing of a repeat blood sample is not required. This is a legal requirement for sperm donations in the EU.

It is recommended that the same testing approach be used for oocyte donors, allowing for the safe use of cryopreserved oocytes (after quarantine and retesting after 180 days) or fresh oocytes (if NAT is done at the time of donation). Oocyte donation should be considered as starting at the first day of stimulation, and the sample for testing should be taken at that time.

25.5. Collection

25.5.1. Sperm

25.5.1.1. *Ejaculated sperm*

Semen collection is the process of obtaining an ejaculate for evaluation and/or for use in an ART procedure (IUI, IVF, ICSI). The ejaculate is composed of secretions from the testis, the prostate gland, and the seminal glands. Unless the patient is azoospermic, it contains the spermatozoa necessary to fertilise an oocyte. The sample is used to determine sperm number, motility and morphology.

25.5.1.1.1. *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.

After thorough cleaning of the hands and genital area, semen must be collected into a sterile collection container. Circumstances under which a semen sample is collected and delivered to the laboratory can influence the results of semen analyses. The time that spermatozoa are kept in the ejaculate can reduce their survival, motility and fertilising ability, so the start of diagnostic/therapeutic treatments must be standardised. If the sample can be collected in a special room adjacent to the laboratory there is a significant reduction of the risk for delays during transportation, and for cooling of the sample. 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.

25.5.1.1.2. *Retrograde ejaculation*

In cases of retrograde ejaculation, where the sperm ends up in the urinary bladder after ejaculation, sperm from urine can be collected after voiding, where the urine pH has been increased by bicarbonates.

25.5.1.1.3. *Collection by electroejaculation*

In some patients (e.g. 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. This procedure is carried out in an operating room and is usually carried out by an urologist.

25.5.1.2. *Surgically retrieved sperm*

In patients diagnosed with non-obstructive or obstructive azoospermia, sperm can be retrieved by surgical means from the testis or epididymis. 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 as described above.

25.5.1.2.1. *Collection of sperm from the epididymis*

Microsurgical epididymal sperm aspiration (MESA) is a surgical method for sperm collection if the vasa deferentia are blocked.

Percutaneous epididymal sperm aspiration (PESA) is a variant of this approach. It involves use of a sterile needle to aspirate sperm from the epididymis without a surgical incision. Both approaches typically yield sufficient quantities of sperm for use for ICSI, but not enough for a standard IUI or IVF.

25.5.1.2.2. *Collection of sperm from the testis*

An alternative to sperm collection from the epididymis is collection of sperm from the testis. Testicular sperm extraction (TESE) and possibly tissue removal (testis biopsy) may be the best option for collecting sperm for ART and could be accompanied by a histological study. 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.

25.5.2. **Oocytes**

Oocyte collection for IVF, also known as oocyte retrieval, is the stage in IVF in which oocytes are collected from the ovaries. Before oocyte collection, the patient will be given hormonal treatment to stimulate the growth and maturation of the follicles in the ovaries (controlled ovarian hyperstimulation [COH]). During treatment, the patient is monitored closely to follow the response to hormonal treatment and to assess the risk of OHSS.

Oocytes are collected through trans-vaginal ultrasound-guided ovarian puncture and aspiration of follicular fluid. The procedure can be carried out under local anaesthesia (paracervical block), sedation or general anaesthesia.

Aspiration pressure and temperature should be controlled. The operating theatre where oocyte collection takes place should be adjacent to the IVF laboratory. If oocytes have to be transported to another

centre for processing, validation of temperature in the transport unit is important.

25.5.3. Ovarian tissue

Collection of ovarian tissue is undertaken solely for fertility preservation purposes. This is addressed in Chapter 26, which will be fully developed in next editions of this Guide.

25.6. Processing

Safety and quality issues covered in Chapter 7 also apply to processing of human gametes and embryos. However, there are some specific issues that must be taken into consideration.

The following section is based mainly on *Revised guidelines for good practice in IVF laboratories* by ESHRE [2]. These guidelines were drawn up by the Special Interest Group (SIG) in Embryology and published in 2008. They constitute the minimum requirements for any laboratory offering ART.

25.6.1. Facilities for processing of gametes and embryos

25.6.1.1. *Laboratory design*

The ART laboratory must have adequate space and should be as close as possible to the operating room in which clinical procedures are carried out. Laboratory construction must ensure aseptic and optimal handling of gametes and embryos during all phases of treatment. Gametes and embryos are sensitive to adverse air quality, so high-efficiency filtration of particulates and volatile organic compounds in the air supplied to the laboratory and rooms in which clinical procedures are carried out should be considered (see Table 25.1).

Table 25.1. Criteria to be considered to determine the air-quality specifications of the processing facilities according to EuroGTP guidance

Criterion	Specific to assisted reproductive technologies
Risk of contamination of tissues or cells during processing	Although very rare, accidental contamination during processing might occur. Working under oil in the laminar flow hood minimises this risk.
Use of antimicrobials during processing	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 and in non-partner oocyte donation. In non-partner sperm donation, 5–10% of final-batch destruction testing is plausible. However, in ART, preliminary microbiological testing of donors before procurement and the methodology of processing are more important.
Risk of transfer of contaminants at transplantation	In ART, only cells in a minimum amount of culture media are transferred in the uterus, so the risk of contamination is very low.

25.6.1.2. *Laboratory equipment*

Incubators in which gametes and embryos are cultured should be organised to facilitate their identification. A minimum of two incubators is recommended, but the number of incubators should be related to the number of cycles to be carried out to avoid unnecessary opening of doors and to maintain stable culture conditions.

Devices for the maintenance of a constant temperature during manipulation of gametes and embryos if they are out of incubators must be in place (i.e. warm stages, heating blocks). Regular checks of critical parameters such as temperature and CO_2 levels must be carried out.

25.6.2. Handling of gametes and embryos

Written standard operating procedures (SOP) for handling of gametes and embryos should be developed and maintained. Procedures should be easy, simple and effective and, if technically possible, carried out

in a laminar flow hood equipped with heating stages and pre-warmed heating blocks. Certain processes, such as ICSI and embryo biopsy, can be done outside the laminar hood because they need to be undertaken under an inverted microscope. Class-II hoods should be used for documented contaminated samples (e.g. HIV, HCV) because they provide protection to the operator. Aseptic methods must be used at all times.

Appropriate measures must be taken to ensure that oocytes and embryos are maintained at 37 °C during handling/observation and, if possible, tissue culture-grade disposables should be used. Pipetting devices: must be used for one type of procedure only; must never be used for more than one patient; should be disposed of immediately after use.

Each sample must be handled individually and its processing should be completed before moving to the next sample.

Labelling of dishes/tubes containing gametes and embryos must be permanent and on the container itself (not only on the removable lid). During labelling, identifying information marked on the culture dish/tube must be cross-referenced to the patient and the patient's documentation. Procedures must be in place that ensure correct identification of patients at all stages of handling, and comprise at least two points of identification (e.g. surname, name, colour code, date of birth). At critical points (e.g. oocyte insemination, embryo transfer, IUI), procedures must be in place to ensure correct identification and matching of gametes and embryos with their corresponding donors/recipients. Identity checking should be done by at least two individuals.

25.6.2.1. *Culture media*

Specific culture media that fulfil the requirements of gametes and embryos are needed during all handling, fertilisation, culture, cryopreservation and other processing activities in ART. Use of commercially produced, quality control-tested media is recommended. If in-house prepared culture media are to be used, they must be

prepared by a validated procedure and at least tested for sterility, pH, osmolarity and endotoxicity. Serum or follicular fluid from donors is not recommended as a medium additive.

Gametes and embryos are often handled and usually cultured under equilibrated mineral oil (for better maintenance of temperature, osmolarity and pH). Hence, it is essential to use only commercially produced oils with documentation of quality-control testing (including an adequate bioassay system) provided by the manufacturer. Each batch of culture media and mineral oil should be recorded in each patient's worksheet or, alternatively, documented during the exact period of time it has been used, thereby making it traceable for each step of the procedure for each patient.

25.6.2.2. *Oocyte processing*

Follicular aspirates should be checked for oocyte–cumulus complexes under a stereo dissecting microscope with a transmitted illumination base and heated stage, usually at a magnification of ×8–60. Exposure of oocytes to light must be minimised. Morphological criteria for the description of the quality and maturity of oocytes, as well as the modality of observation, must be specified. Evaluation of recovered oocytes must be documented in the patient's worksheet.

25.6.2.3. *Sperm processing*

The method of processing is chosen according to individual samples. A trial sperm preparation before the treatment cycle may be advisable to choose the most adequate method. Sperm processing aims to:

a. concentrate and select active and motile sperm;

b. discard seminal plasma, debris and contaminants;

c. separate and discard the most abnormal forms.

Different methods are used for sperm processing. Among them, the swim-up method and discontinuous density-gradient centrifugation are used most frequently. A record must be kept of pre- and post-preparation sperm parameters and of dilution carried out before insemination.

25.6.3. Insemination of oocytes

25.6.3.1. *Conventional in vitro fertilisation*

In conventional IVF, oocytes are inseminated with processed sperm usually 2–5 h after retrieval (38–42 h after triggering of ovulation). A sufficient concentration of motile sperm in the insemination suspension is needed to achieve an insemination volume as low as possible (usually ≈100,000 sperm/ml of culture medium).

25.6.3.2. *Intracytoplasmic sperm injection procedure*

a. Preparation of oocytes for ICSI by removal of cumulus–corona cells

Oocytes are denuded from the surrounding cumulus and corona cells using an enzymatic procedure with hyaluronidase, often followed by mechanical denudation using a calibrated pipette. Care must be taken to avoid damage to oocytes.

b. Injection

The success of ICSI is dependent upon the: morphology and maturity of each oocyte; selection and immobilisation of viable sperm; rupture of oocyte membranes before release of sperm into the oocyte.

It is important to select vital spermatozoa, as evaluated by their motility. In case of only immotile sperm, a vitality test can be used to select vital spermatozoa at the time of injection. If only non-vital sperm are present in the ejaculate, use of mature testicular sperm may be an alternative.

In any case, only mature metaphase II (MII) oocytes should be used for injection.

25.6.4. Assessment of fertilisation

All oocytes that have been inseminated or microinjected must be examined for signs of fertilisation (presence and number of pronuclei and polar bodies) 16–20 h after insemination. This examination should be done under high magnification (at least ×200) using an inverted microscope equipped with Hoffman optics or equivalent. The

morphological status of each zygote should be recorded. Zygotes with three or more pronuclei must not be used for transfer.

25.6.5. Embryo culture and transfer

Pre-implantation embryos are sensitive to culture stress, which may result in perturbed metabolism with altered cell function, energy production and gene expression [3]. Therefore, precautions must be taken to maintain adequate conditions of pH, osmolarity and temperature to protect embryo homeostasis.

Embryo assessment should be done at high magnification (at least ×200 but preferably ×400) under an inverted microscope with Hoffman optics or equivalent. Evaluation should include (but not necessarily be limited to): number of cells; percentage of fragmentation; size and cytoplasmic appearance of blastomeres; recording of nuclear status (presence of one or several nuclei per blastomere). Dynamic observation of embryo development by a time-lapse system can also be used.

Embryos can be cultured and transferred up to the blastocyst stage (5–7 days of development). Supernumerary embryos may be cryopreserved for later use. According to national legislation and the couple's wish (stated in a specific informed consent form) cryopreserved embryos may be used for the transfer, research, donation of embryos, or be discarded.

The stage of embryo development at the time of transfer should be documented. In some countries, the maximum number of embryos to be transferred is established by local/national legislation. It is advisable not to exceed two embryos for transfer. In cases in which two or more embryos are transferred, the recipient must be informed comprehensively on the risks of multiple pregnancy. As a general recommendation, the policy of single-embryo transfer is highly recommended. Single-embryo transfers are now mandatory in some countries and practiced to a high extent in other countries.

25.6.6. Pre-implantation genetic diagnosis and pre-implantation genetic screening

Oocytes and pre-implantation embryos can be biopsied and the obtained genetic material tested for certain monogenic disorders or chromosomal abnormalities. The biopsy procedure may be carried out by:

a. removal of polar bodies;

b. single or double blastomere biopsy at day 3;

c. trophectoderm biopsy at the blastocyst stage.

Cell(s) destined for genetic investigation 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 the operator. They must be identified and labelled carefully, and tracked during the entire procedure. During these steps, double identity checks are strongly recommended. The biopsy sample should be subjected to diagnostic procedures in a genetic laboratory.

The purpose of PGD is to identify embryos that have been generated *in vitro* that carry certain hereditary genetic disease or chromosomal abnormalities and exclude them from transfer. PGD is an alternative to therapeutic abortion due to a minimised risk of transferring affected embryos.

Genetic counselling should be available to all couples known to carry a hereditary disease.

PGS is carried out in the same way as PGD. PGS is used to check if an embryo has the correct number of chromosomes (also known as 'pre-implantation aneuploidy screening') if there is no hereditary genetic indication. 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. Bearing in mind the conflicting data from prospective clinical trials and meta-analyses, PGS should be offered with caution and full information on its present value should be provided to the patients.

In some countries, PGD and/or PGS are not allowed or allowed only in specific circumstances according to national legislation.

25.6.7. *In vitro* maturation

In vitro maturation (IVM) refers to the maturation in culture of immature oocytes in special media after recovery from follicles that may or may not have been exposed to exogenous gonadotropins before retrieval. During IVM, such oocytes progress from prophase I (i.e. from germinal vesicle [GV]) to reach metaphase II (MII) [4]. 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 full information on its present value should be provided to patients.

25.6.8. Processing of samples from seropositive donors

25.6.8.1. *Non-partner donation*

If initial testing for transmissive infections proves a donor to be positive, he/she must be excluded from the donor programme.

25.6.8.2. *Partner donation*

In couples with one or both partners being seropositive, ART 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.

Discordant couples with seropositive males, if fallopian tubes are patent, should be treated by artificial insemination with specifically processed semen. The process includes density-gradient separation

and optional swim-up. This procedure does not completely eliminate the risk but reduces it to minimal levels [5,6]. If the infertility factor requires IVF, ICSI should be preferred [7]. In HIV-positive male donors, a nested polymerase chain reaction (PCR) assay for HIV-1 RNA (RT-PCR) and proviral DNA is recommended before insemination [8, 9] of the female partner or oocytes.

Discordant couples with seropositive females, if fallopian tubes are patent, should be treated by artificial insemination (or self-insemination at home after appropriate counselling).

Processing of samples from seropositive partner donors should be handled according to specific SOP to protect personnel and avoid cross-contamination.

Hepatitis B-seronegative females with seropositive partners should be offered vaccination before ART.

25.7. Cryopreservation and storage

25.7.1. Methods for cryopreservation of human gametes and embryos

Sperm, embryos and, more recently, oocytes are being cryopreserved for future use in ART (supernumerary embryos or oocytes, fertility preservation, non-partner donor gametes for quarantine). At present, the two most used methods for freezing gametes and embryos are slow freezing and vitrification.

1. Slow freezing is a method for programmed step-wise decreases in the temperature of the solution in which the specimen is cryopreserved. This involves specific computerised equipment and programmes for cooling different types of tissues and cells in solutions with cryoprotectant substance(s).
2. Vitrification is an ultra-rapid cooling method that relies on very fast temperature drops (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. This is a fast method that does not require special

equipment (but special consumables are required) with the addition of specific cryoprotectants in higher (rather than in slow freezing) concentrations for shorter exposure times.

Concerns about the safety and quality of cryopreserved human gametes and embryos are raised:

a. cell damage (disassembly of meiotic spindles, membrane rupture);

b. toxic effects of cryoprotectants (anti-freeze substances):

   i. general (osmotic damage, dehydration);

   ii. specific (effects of the toxicity of individual compounds on cell viability).

However, there are significant differences in the sensitivity of different types of male and female gametes and different-stage embryos towards the cooling process and cryoprotectant agents used.

25.7.1.1. *Cryopreservation of sperm*

Freezing/thawing of human sperm is a well-proven technology. Sperm samples are usually cryopreserved in glycerol-based cryoprotectant solutions in cryovials or straws on liquid nitrogen vapours and then plunged in the liquid phase. There have been reports suggesting that semen quality could deteriorate after freezing/thawing [10] . However, the results for the last 50–60 years have been good, with hundreds of thousands of children born without significant abnormalities compared with the general population. Clinical results have not revealed dramatic biological hazards [11].

Immotile and damaged sperm could be removed (by sperm preparation) before freezing to select a population of sperm with a better chance of survival. It is recommended to process testicular biopsy samples before freezing.

25.7.1.2. *Cryopreservation of oocytes*

In recent years, successful cryopreservation of MII human oocytes has been reported worldwide with rapidly increasing success rates due to

optimisation of protocols. These data suggest that vitrification may be the method of choice in oocyte cryopreservation because higher rates of survival, implantation and pregnancy have been obtained using this method [12, 13].

25.7.1.3. *Cryopreservation of embryos*

Zygotes, early-cleavage embryos, morulae and blastocysts have been cryopreserved successfully and used later for 'frozen embryo transfer'. Slow-freezing and vitrification can be used [14, 15], with vitrification/warming in dimethyl sulfoxide-based cryoprotectants resulting in better survival rates. Exposure time to the cryoprotectant before vitrification is crucial and must be strictly respected.

25.8. Storage

25.8.1. 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 [16]. Use of frozen sperm through assisted reproduction techniques has led to the birth of healthy offspring more than 20 years after initial storage [17, 18]. 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 have determined 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 section 25.2 Consent).

25.8.2. Storage temperature

Storage in liquid nitrogen or liquid nitrogen-vapours is common practice. Optimal storage temperature is based on the type of tissue, cryoprotectant, and freezing method used. However, a temperature less than –136 °C for gonadal tissue, embryos and gametes is appropriate,

and higher than –130 °C is detrimental for the survival and quality of the material frozen.

25.8.3. 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.

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.

25.8.4. Cross-contamination during storage

Introduction of contamination in the storage vessel is due to human manipulations during processing. Viral and microbial agents may survive during long periods of time in liquid nitrogen. However, no reports have shown cross-contamination of these environmentally induced pathogens to the preserved reproductive material. Also, storage of reproductive material originating from patients carrying infectious diseases in liquid nitrogen has not led to cross-contamination of other frozen reproductive material residing in the same vessel. Even though evidence is lacking [19], it should be considered good laboratory practice to store reproductive material of patients with positive serology and negative serology separately. Vapour phase storage containers have been proposed as an alternative to liquid nitrogen containers. Periodic thawing and cleaning of storage vessels is recommended for extending the lifetime of the vessel as well as periodic decontamination of viral and microbial agents.

25.8.5. Storage safety

Storage in liquid nitrogen or vapour nitrogen vessels is definitely the most common infrastructure used to store gametes, embryos and gonadal tissue. Cryopreservation and thawing of material is a daily process in each ART centre. Therefore, it is of utmost importance that personnel working in the cryogenic room have received appropriate training on how to handle liquid nitrogen, and that they are aware of the potential hazards. Personnel must be equipped with specific protective garment (gloves, boots, goggles) and use special forceps for manipulation of straws.

25.9. Packaging and labelling in assisted reproductive technologies

Packaging applies only to cryopreserved gametes and embryos in storage and transport. Frozen gametes and embryos are packaged and stored in straws/cryovials as described in section 25.7.1.1.

Labelling is intended to identify gametes and embryos unambiguously. Labelling and identification systems may vary between centres and countries As mentioned in section 25.6.2, procedures must be in place that ensure correct identification of patients at all stages of handling, comprise at least two points of identification (e.g. surname, name, colour code, date of birth) and should include at least the names of partners and date of processing. For frozen samples, colour coding of cryovials and straws could also be used.

Tissue establishments that store and distribute non-partner gametes should label containers with an appropriate unique donation identification code. In the EU, the coding requirements for non-partner donation apply (see Chapter 12).

25.10. Vigilance in assisted reproductive technologies

Vigilance in ART involves not only vigilance in terms of tissues and cells, but also pharmacovigilance (e.g. OHSS) and vigilance in terms of medical devices (e.g. media, incubators, aspiration needles).

In ART, the following specific characteristics of reproductive cells/embryos must be considered:

a. oocytes and embryos are available in (very) limited numbers;

b. reproductive cells are particularly sensitive to external factors (temperature, culture media, laboratory equipment, pollutants);

c. any defect does not only have an impact on the recipient of cells but also affects at least one more individual (offspring);

d. adverse outcomes are, in general, associated with a loss of gametes or embryos and subsequent loss of chance of pregnancy (unlike other tissues or cells: illness, disability).

Specific aspects of ART vigilance should (among others) focus on:

a. sensitivity of gametes/embryos (impact of equipment, media and environment);

b. traceability;

c. mix-ups;

d. complications during procurement;

e. cross-border management of serious adverse events and reactions (SARE).

Serious adverse events (SAE) and serious adverse reactions (SAR) are defined in Directive 2004/23/EC for the EU. Mis-identifications, mix-ups as well as total loss of reproductive cells, germinal tissues and embryos for one cycle should be considered SAE in the context of ART.

The definition for SAR in the context of ART should be extended to the offspring in non-partner donation for transmission of genetic diseases (see section 25.10.2).

25.10.1. General criteria for reporting serious adverse reactions and events in assisted reproduction technologies

In ART vigilance, deviations from SOP 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 if one or more of the following criteria apply:

a. inappropriate gametes, embryos, or germinal tissues have been released for clinical use (even if they are not used);

b. the event could have implications for other patients or donors because of shared practices, services, supplies, critical equipment or donors;

c. the event resulted in a mix-up of gametes or embryos;

d. the event resulted in a loss of traceability of gametes or embryos;

e. contamination or cross-contamination;

f. accidental loss of gametes, embryos, germinal tissues (e.g. breakdown of incubators, accidental discards, manipulation errors) resulting in a total loss of chance of pregnancy for one cycle.

Mix-ups result from an error in the attribution of reproductive cells or embryos which can occur at any stage of clinical or laboratory processes (collection, insemination, embryo transfer, cryopreservation). Reasons for mix-ups (e.g. multiple processing steps, mislabelling, too many people involved, misidentification, over-worked staff, absence/failure of witnessing, poor-quality systems) as well as the consequences of mix-ups (loss of traceability, loss of gametes or embryos, disease transmission, psychological impact, ethical/legal issues) are diverse and should be reported to the national Health Authority immediately no matter at which stage they were detected. Causal factors should be investigated.

All patients involved should be informed as soon as possible and should be offered counselling and support.

Hospitalisation of oocyte donors for observation should be considered as a non-serious adverse reaction.

In the EU, Health Authorities must be notified without delay of all SARE which may influence the quality and safety of tissues and cells, and which may be attributed to the procurement, testing, processing, storage and distribution of tissues and cells as well as any SARE ob-

served during or after clinical application which may be linked to the quality and safety of tissues and cells. Even though it is not mandatory in all EU member states, it is highly recommended that Health Authorities should be notified of any SARE involving harm to donors.

All SARE related to stimulation and procurement (especially severe OHSS) should be reported to Health Authorities. Coordination between various systems of vigilance (tissues and cells, pharmacovigilance, medical-device vigilance) should be established by Health Authorities.

25.10.2. Transmission of genetic diseases by assisted reproductive technologies with non-partner donations

Non-partner donors may unknowingly carry genetic defects. Sperm banks, for example, could supply sperm from a donor to multiple recipients, thereby spreading genetic disease. Tissue establishments should keep in mind genetic diseases when educating non-partner donors as well as recipients of non-partner donations. National registries of gamete donors 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. Recipients of non-partner donations should be advised to inform 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. If necessary, the distribution of gametes from that donor could be discontinued.

25.10.3. Examples of serious adverse reactions and events in assisted reproduction technologies

The Notify Library includes many well-documented cases of adverse occurrences in ART.

- A case of spinal muscular atrophy in a child born from an embryo created by an anonymous oocyte donor and an anonymous sperm donor. It was confirmed by molecular genetic inves-

tigations that both anonymous donors were carriers of one copy of the SMN1 deletion mutation (Record Number 92).

- A case of embryo mix-up detected after embryo transfer that resulted in a medical abortion (Record Number 978).
- A serious adverse event in which embryo-toxic oil was used during culture that led to degeneration of 11 embryos from three couples (Record Number 981).

For further evaluated cases of adverse outcomes in ART, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance, adverse occurrence, and Record number.

25.10.4. 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 SARE might occur and do not get reported to the professionals who carried out the treatment and 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 SARE to their national Health Authorities even for cross-border treatments.

25.11. Final considerations

Fair, clear and appropriate information must be provided at all stages of ART treatment. The chances of success (including the take-home baby rate) should be discussed appropriately. Clinicians, embryologists, technicians, nursing staff and all professionals need to communicate frequently 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 be addressed.

25.12. References

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Chapter 26

Fertility preservation[1]

Advances in the diagnosis and treatment of malignant diseases, the increased life expectancy of patients being treated for cancer, and the fruitful collaboration between oncologists and specialists in reproductive medicine has allowed development of the new area of fertility preservation.

It is well established that oncologic treatments such as chemotherapy and radiotherapy may compromise future fertility in infants and young adults because of the effect of such therapies in germinal tissues. This effect has a direct relationship with the age of the patient. Different options can be considered to preserve fertility in both sexes.

In the case of preservation of female fertility, oocyte cryopreservation should be considered as the most realistic option. Oocyte vitrification has proved to be an effective method, and results similar to those obtained with fresh oocytes have been published extensively.

In pre-pubescent females, or if hormonal treatment is contraindicated, or if there is no time for hormonal stimulation, freezing of

1 This electronic edition has been corrected to remove some duplicated material in Chapter 26, discovered after the print edition had gone to press.

ovarian tissue is the preferred option. Subsequent transplantation of thawed cortices of ovarian tissue may restore ovarian function (including ovulation). Increasing numbers of pregnancies are being reported by freezing and transplantation of ovarian tissue by means of spontaneous pregnancies or in vitro fertilisation. However, bearing in mind the limited number of reported cases, full information of its present value should be given to the patients.

Preservation of female fertility can also be considered for non-malignant or genetic diseases that may impair reproductive function (e.g. endometriosis; Turner Syndrome) and for women anticipating haematopoietic stem cell transplantation for non-malignant haematologic diseases or affected by autoimmune diseases.

Age-related preservation of female fertility through oocyte vitrification is becoming an option for young women who would like to postpone maternity.

In the case of preservation of male fertility, spermatogenesis seems to be less affected by anti-neoplastic treatments. Sperm cryopreservation is the option of choice whenever a sperm sample can be obtained. In the case of male infants, cryopreservation of testicular tissue should be considered even though the method is experimental and no conclusive proof of restoration of spermatogenesis has been reported in humans.

It is also possible for other patient populations, like transsexual persons, to benefit from fertility preservation techniques although the use of the preserved tissue and cells is questionable and even debatable.

In recognition of the importance and specificity of fertility preservation as a component of the large and heterogeneous field of assisted reproductive technology, dedicated safety and quality standards along with ethical guidance should be elaborated for this new area. This will be included in the third edition of the *Guide to the quality*

and safety of tissues and cells for human application (to be published in 2017).

Appendix 1

General reference documents used

The experts who developed the chapters in this Guide incorporated principles and specific text from many regulatory, professional, and scientific publications. The following are the most important reference documents used.

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- Council of Europe (1997) Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, available at http://conventions.coe.int/Treaty/en/Treaties/Html/164.htm, accessed 27 May 2015.
- Council of Europe (2002) Additional protocol to the Convention on Human Rights and Biomedicine, on Transplantation of Organs and Tissues of Human Origin, available at http://conventions.coe.int/Treaty/en/Treaties/Html/186.htm, accessed 27 May 2015.
- Council of Europe 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 27 May 2015.
- Council of Europe Resolutions, Recommendations and Reports related to Safety, Quality and Ethical Matters concerning Procurement, Storage and Transplantation of Organs, Tissues and Cells, available at www.edqm.eu/site/booklet_rec_res_organ_transplantationpdf-en-30783-2.html, accessed 27 May 2015.
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Appendix 2

Acronyms

AATB	American Association of Tissue Banks
AAV	Adeno-associated virus
Ab	Antibodies
ACI	Autologous chondrocyte implantation
AFC	Antral follicle count
AIDS	Acquired immunodeficiency syndrome
hAM	Human amniotic membrane
ALK	Anterior lamellar keratoplasty
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 reactions and events

ART	Assisted reproductive technology
ATMP	Advanced therapy medicinal products
aW	Available water
BET	Bacterial endotoxin test
BMP	Bone morphogenetic proteins
BMSC	Bone marrow stromal cells
BSS	Buffered salt solution
CAT	Committee for Advanced Therapies
CBC	Complete blood count
CD	Cell-surface antigens
CD-P-TO	European Committee (Partial Agreement) on Organ Transplantation of the Council of Europe
CDC	Centers for Disease Control and Prevention
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-propanesulfonate
CJD	Creutzfeldt–Jakob disease
CLET	Cultivated limbal epithelial transplantation
CMV	Cytomegalovirus
CNS	Central nervous system
CNT	Centro Nazionale Trapianti (Italy)
COD	Cause of death
COHS	Controlled ovarian hyperstimulation
COMET	Cultivated oral mucosal epithelial transplantation

DBM	Demineralised bone matrix
DC	Dendritic cells
DBO	Department of Biological Standardisation, OMCL Network & HealthCare (at the European Directorate for the Quality and Medicines & Healthcare)
DH-BIO	Committee on Bioethics of the Council of Europe
DLI	Donor lymphocyte infusions
DM	Diabetes mellitus
DMEK	Descemet membrane endothelial keratoplasty
DMSO	Dimethyl sulfoxide
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
ECDC	European Centre for Disease Prevention and Control
ECM	Extracellular matrices
ECVAM	European Centre for the Validation of Alternative Methods
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	Ethylenediamine tetra-acetic acid
EGF	Endothelial growth factor
EGTA	Ethylene glycol tetra-acetic acid

EIA	Enzyme immunoassay
EMA	European Medicines Agency
EQSTB	European Union project 'European Quality System for Tissue Banking'
ESBL	Extended-spectrum beta lactamases
ESHRE	European Society for Human Reproduction and Embryology
EU	European Union
Euro-GTP	European Union project 'Euro-Good Tissue Practices'
EUSTITE	European Union project 'European Standards and Training in the Inspection of Tissue Establishments'
EUTC	European Code for Tissues and Cells
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration (USA)
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
GF	Growth factors
GMP	Good manufacturing practice
GM-CSF	Granulocyte macrophage-colony stimulating factor
GPA	Glycerol-preserved allografts

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 leucocyte antigen
hMG	Human menopausal gonadotrophin
HPV	Human papilloma virus
HRV	Human rotavirus
HSC	Haematopoietic stem cell
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

ICCBBA	International Council for Commonality in Blood Banking Automation
ICSI	Intra-cytoplasmic sperm injection
ICU	Intensive care unit
IEC	Independent Ethics Committee
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IQ	Installation qualification
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineering
ISCT	International Society for Cellular Therapy
ISN	International Society for Nephrology
ISSCR	International Society for Stem Cell Research
IT	Information technology
IUI	Intra-uterine insemination
IVF	In vitro fertilisation
IVM	In vitro maturation
KIR	Killer immunoglobulin-like receptors
LAL	Limulus amoebocyte lysate
LH	Luteinising hormone
LSC	Limbal stem cells
MII	Metaphase II
MESA	Microsurgical epididymal sperm aspiration
MPHO	Medical products of human origin
MRSA	Staphylococcus aureus
MRA	Marrow re-populating ability

MRSA	Methicillin-resistant Staphylococcus aureus
MSC	Mesenchymal stem cells
NAT	Nucleic acid amplification technique/nucleic acid test
NEC	Necrotising enterocolitis
NICE	National Institute of Health and Clinical Excellence
NK	Natural killer
OA	Osteoarthritis
OECD	Organisation for Economic Co-operation and Development
OHSS	Ovarian hyper-stimulation syndrome
ONT	Organización Nacional de Trasplantes (Spain)
OQ	Operational qualification
ORHA	Organisations responsible for human application
Parvo-B19	Parvovirus B19
PCR	Polymerase chain reaction
PESA	Percutaneous epididymal sperm aspiration
PGD	Pre-implantation genetic diagnosis
PGS	Pre-implantation genetic screening
Ph. Eur.	European Pharmacopoeia
PIC/S	Pharmaceutical Inspectorate Co-operation Scheme
PO	Procurement organisation
POSEIDON	European Union project 'Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe'
PQ	Performance qualification

PRIVILEGED	European Union project 'Privacy in Law, Ethics and Genetic Data'
PRF	Platelet-rich fibrin
PROH	1,3-propanedial
PRP	Platelet-rich plasma
QC	Quality control
QM	Quality manager
QMS	Quality management system
QRM	Quality risk management
RANTES	Regulated on activation, normal T cell expressed and secreted
RATC	Rapid alerts for tissues and cells
RBC	Red blood cell
RhD	Rhesus D antigen
rhG-CSF	Recombinant granulocyte-colony stimulating factor
RP	Responsible person
RPN	Risk priority number
SAE	Serious adverse event
SAR	Serious adverse reaction
SP-CTO	Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation
SAL	Sterility assurance level
SAR	Serious adverse reaction
SARE	Severe adverse reactions and events
SDS	Sodium dodecyl sulfate
SEC	Single European Code
SIG	Special interest group

SOHO V&S	Vigilance and Surveillance of Substances of Human Origin
SOP	Standard operating procedure
S(P)EAR	Serious (product) events and reactions
T1DM	Type-1 diabetes mellitus
TBV	Total blood volume
TE	Tissue establishment
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
TSE	Transmissible spongiform encephalopathy
TTS	The Transplantation Society
vCJD	Variant Creutzfeldt–Jakob disease
VEGF	Vascular endothelial growth factor
V&S	Vigilance and surveillance
VMP	Validation master plan
VRE	Vancomycin-resistant enterococci
WHO	World Health Organization
WMDA	World Marrow Donor Association
WNV	West Nile virus
3D	Three-dimensional

Appendix 3

Glossary

Acceptance criteria	The standards required to satisfy quality and safety expectations and ensure an acceptable quality and safety of the final product.
Advanced therapy medicinal product	A medicinal product that is either a gene therapy medicinal product, a somatic cell therapy medicinal product, a tissue-engineered product, or a combined advanced therapy medicinal product (which are medicinal products incorporating cells and medical devices or actively implantable medical devices).
Adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells.
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.

Allogeneic	Refers to tissues and cells donated by one person for clinical application to another person.
Allograft	Tissues or cells transplanted between two genetically different individuals of the same species. The term is synonymous with the term 'homograft'.
Ambient temperature	The temperature of the surrounding environment. In temperature-controlled facilities, ambient temperature equals the room temperature and is usually 17–21 °C for thermal comfort.
Amniotic membrane	The innermost layer of the placental membrane; it surrounds the foetus during pregnancy.
Apheresis	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.
Artificial insemination	See 'Intrauterine insemination'.
Assisted hatching	An in vitro procedure in which the zona pellucida of an embryo is either thinned or perforated by chemical, mechanical or laser methods to assist separation of the blastocyst.

Assisted reproductive technology	All treatments or procedures that include the in vitro handling of human oocytes and sperm 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 bodies.
Autologous	Refers to tissues or cells removed from a patient for subsequent clinical application to themselves.
Azoospermia	Absence of spermatozoa in the ejaculate.
Banking	Processing, preservation, storage and distribution of tissues and cells for therapeutic and/or research purposes.
Barcode	An optical machine-readable representation of data relating to the object to which it is attached.
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 homogenous.

Best practice	A method or technique that has consistently shown results superior to those achieved with other means and which is used as a benchmark.
Biobank	A collection of biological material and the associated data and information stored in an organised system for a population or a subset of a population.
Bioburden	Total number of viable micro-organisms (total microbial count) on tissues or cells or on the environment, usually measured before the application of a decontamination or sterilisation process.
Blastocyst	An embryo, 5 or 6 days after fertilisation, with an inner cell mass, outer layer of trophectoderm, and a fluid-filled blastocoele cavity.
Blastomere	Undifferentiated cells formed after cleavage of the fertilised oocyte (zygote).
Bone	The hard, rigid 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 cells: 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	The result of in vitro cell growth.
Clean area/ environment	An area with defined environmental control of particulate and microbial contamination constructed and used in such a way as to reduce the introduction, generation and retention of contaminants within the area.
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 automatic control.
Consent to donation	Legally valid permission or authorisation for removal of human cells, tissues and organs for transplantation.
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 stem cells.
Cord blood bank	A specific type of tissue establishment in which haematopoietic stem cells collected from the placental and umbilical cord blood vessels are processed, cryopreserved and stored. It may also be responsible for collection, testing or distribution.
Cornea	The transparent dome-shaped anterior portion of the outer covering of the front of the eye; it covers the iris and pupil and is continuous with the sclera.
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	Freezing or vitrification and storage of gametes, zygotes, embryos or gonadal tissue.
Culture/cell expansion	In vitro proliferation of retrieved cells for the purpose of transplantation.
Cumulative delivery rate with at least one live born baby	An estimated number of deliveries with at least one live born baby resulting from one initiated or aspirated ART cycle, including the cycle when fresh embryos are transferred, and subsequent frozen/thawed ART cycles. The delivery of a singleton, twin or other multiple pregnancies is registered as one delivery.

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 transplantation. Possible medical criteria are:

- Deceased Heart-Beating Donor (Donor after Brain Death): a donor who is declared dead and diagnosed by means of neurological criteria.
- Deceased Non-Heart-Beating Donor (Donor after Cardiac Death) (NHBD): a donor who is declared dead and diagnosed by means of cardiopulmonary criteria.

Decontamination See 'Disinfection'.

Delivery rate Number of deliveries expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles. When delivery rates are given, the denominator (initiated, aspirated or embryo transfer cycles) must be specified. It includes deliveries that resulted in the birth of one or more live babies and/or stillborn babies. Delivery of a singleton, twin or other multiple pregnancies is registered as one delivery.

Delivery rate after ART treatment Number of deliveries with at least one live born baby per patient following a specified number of ART treatments.

Design qualification The first step in the qualification of new equipment or facilities.

Deviation Departure from an approved instruction or established standard.

Direct use	Any procedure in which tissues and cells are donated and used without banking/storage.
Discontinuous gradient centrifugation	Sperm-preparation technique based on sedimentation of sperm at different rates depending on motility.
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 or cells)	The act or means of discarding tissues and cells.
Distribution	Transportation and delivery of cells or tissues intended for human application after they have been allocated.
Donor	A person, living or deceased, who is a source of tissues or cells for the purpose of transplantation.
Donor evaluation	The procedure of determining the suitability of a potential donor, living or deceased, to donate.
Donor selection	See 'Donor evaluation'.
Double-embryo transfer	Transfer of two embryos, selected from a larger cohort of available embryos.
Egg sharing	A female partner who enters an ART treatment decides to donate a specified number of her retrieved oocytes.
Elective embryo transfer	Transfer of one or more embryos selected from a larger cohort of available embryos.
Elective single-embryo transfer	Transfer of one embryo selected from a larger cohort of available embryos.

Embryo	Product of the division of the zygote to the end of the embryonic stage 8 weeks after fertilisation. (This definition does not include parthenotes – generated through parthenogenesis – or products of somatic cell nuclear transfer.)
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.
End user	A healthcare practitioner who undertakes transplantation procedures.
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.
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.
Expert	Individual with 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.
Export	Act of transporting human bodies, body parts, tissues or cells intended for human application to another country where they are to be processed further or used.

Facility	Refers to a physical building or part of a building.
Fascia	A layer of fibrous connective tissue that surrounds muscles, groups of muscles, blood vessels and nerves, which binds some structures together while permitting others to slide smoothly over each other.
Fertilisation	Penetration of the ovum by the spermatozoon and combination of their genetic material resulting in the formation of a zygote.
Fertility preservation	Long-term cryopreservation of reproductive tissues and cells before treatments that may be potentially deleterious for reproductive cells.
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 transplantation, maintenance of care and initiating post-donation or post-transplantation interventions.
Gamete intrafallopian transfer	ART procedure in which both gametes (oocytes and spermatozoa) are transferred to the Fallopian tubes.
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	A 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.
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.
Haematopoietic stem 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. May also be referred to as 'Haematopoietic Progenitor Cells'.
Haemodilution	Dilution of serum or blood samples used for laboratory investigations due to infusions and transfusions.
Hatching	Process by which an embryo at the blastocyst stage separates from the zona pellucida.
Health Authority	In the context of this Guide, the body which has been delegated the responsibility for ensuring that tissue and cell donation, banking and human application are appropriately promoted, regulated and monitored in the interest 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 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.
Human application	Use of tissues or cells on or in a human recipient, and extracorporeal applications.
Human error	A mistake made by a person rather than being caused by a poorly designed process or malfunctioning of a machine such as a computer.
Human tissues and cells for transplantation	Material containing or consisting of human tissues and/or cells intended for implantation, transplantation, infusion, or transfer into a human recipient. Examples include, but are not limited to, musculoskeletal tissue (bone, cartilage, meniscus), skin, soft tissue (tendons, ligaments, nerves, dura mater, fascia lata and amniotic membrane), cardiovascular tissue (heart valves, arteries and veins), ocular tissue (corneas and sclera), bone marrow, HSCs derived from peripheral and cord blood, stem cells from any tissue and reproductive tissues and cells.
Identification of tissues and cells	The act of labelling tissues and cells to uniquely designate their origin, use or destination.
Implantation	Attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) that starts 5–7 days after fertilisation.
Import	Act of bringing tissues or cells into one country from another for the purpose of transplantation.

Imputability	Assessment of the probability that a reaction in a donor or recipient may be attributed to the process of donation or clinical application or to an aspect of the safety or quality of the tissues or cells applied.
***In vitro* fertilisation**	ART procedure that involves extracorporeal fertilisation.
***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.
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 impacting on patients that is 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.
In-process control	Checks undertaken during processing to monitor and, if necessary, to adjust the process to ensure that the product conforms to its specification. Control of the environment or equipment may also be regarded as a part of in-process control.
Inspection	On-site assessment/control of compliance with the local/national regulations for tissues and cells carried out by officials of the Health Authority(ies).

Installation qualification	The second step in the qualification of new equipment or facilities.
Intra-cytoplasmatic sperm injection	a procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
Intrauterine insemination	Procedure in which processed sperm cells are transferred transcervically into the uterine cavity.
Labelling	Includes steps taken to identify packaged material by attaching the appropriate information to the container or package so they are clearly visible through the immediate carton, receptacle or packaging.
Live birth rate	Delivery of one or more infants with any signs of life per initiated ART cycle.
Living donor	A living person from whom cells or tissues have been removed for the purpose of transplantation. A living donor has one of the following possible relationships with the recipient: A/Related: A1/Genetically related: • First-degree Genetic relative: Parent, Sibling, Offspring. • Second-degree Genetic relative: Grandparent, Grandchild, Aunt, Uncle, Niece, Nephew. • Other than first or second degree genetically related, e.g. Cousin. A2/Emotionally related: Spouse (if not genetically related), in-laws, Adopted, Friend.

	B/Unrelated = Non-related: Not genetically or emotionally related.
Lyophilisation	A controlled dehydration process typically used to preserve a perishable material or to make the material more convenient for transport.
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 transplantation. In the context of processing of haematopoietic stem cells, this is an in vitro procedure that selectively removes, enriches, expands or functionally alters the cells.
Medicinal product	Any substance or combination of substances presented as having properties for treating or preventing disease in human beings, or any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.
Meiotic spindle	Spindle apparatus composed of microtubules that support and segregates chromosomes during meiotic division.
Metaphase II oocyte	Mature oocyte at the metaphase of the second meiotic division.
Micromanipulation	Technology that allows micro-operative procedures to be done on the spermatozoon, oocyte, zygote or pre-implantation embryo.

Microsurgical epididymal sperm aspiration	Aspiration of epididymal sperms as an open operation under the operating microscope.
Musculoskeletal	Tissues that are part of the skeletal and/or muscular system such as muscles, bones, cartilage, tendons and ligaments which function in support and movement of the body.
Next of kin	A person's closest living blood relative or relatives.
Non-compliance	Failure to comply with accepted standards, requirements, rules or laws.
Oligozoospermia	Total number of spermatozoa <15×106/ml.
One-off import	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 the 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' (Commission Directive (EU) 2015/566).
Oocyte cumulus complex	Oocyte surrounded by the granulosa cells and corona radiate.
Operational qualification	Third step in the qualification of new equipment or facilities.
Opt-in donation system	Method for determining voluntary consent where only those who have given explicit consent are donors.

Opt-out donation system	Method for determining voluntary consent where anyone who has not refused donation is a donor.
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.
Organisations responsible for human application	Healthcare establishment or a unit of a hospital or another body which carries out human application of human tissues and 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 distention, ovarian enlargement and respiratory, haemodynamic and metabolic complications.
Ovulation induction	Pharmacological treatment of women with anovulation or oligo-ovulation with the intention of inducing normal ovulatory cycles.
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	All operations, including filling and labelling, in which a tissue or cell product must undergo to become a finished product.

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.
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.
Placenta	An organ that connects the developing foetus to the uterine wall to allow nutrient uptake, waste elimination and gas exchange via the mother's blood supply.
Pooling	Physical contact or mixing of tissues or cells from two or more donors in a single container. Use of any shared space during processing, such as laminar flow cabinets or freeze-dryer chambers, also implies pooling.
Preimplantation genetic diagnosis	Analyses of polar bodies, blastomeres or trophectoderm from oocytes, zygotes or embryos for the detection of specific genetic, structural and/or chromosomal alterations.

Pre-implantation genetic screening	Analyses of polar bodies, blastomeres or trophectoderm from oocytes, zygotes or embryos for the detection of aneuploidy, mutation and/or DNA rearrangement.
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, excluding any outer packaging used for transportation or shipment, intended to be in direct contact with the graft.
Procedures	Description of the operations to be carried out, the precautions to be taken and measures to be applied that relate directly or indirectly to the process from donation to transplantation.
Processing	All operations involved in the preparation, manipulation, preservation and packaging of tissues or cells intended for human application.
Procurement	A process by which tissues or cells are made available for banking or clinical use. 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.
Prophase I oocyte	Immature oocyte at the prophase of the first meiotic division.
Quality	The degree to which a set of characteristics fulfils requirements.

Quality assurance	Describes the planned and performed actions 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 focussed on fulfilling quality requirements. In terms of preparation, it is concerned with sampling specifications and testing and, for the 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	Describes the planned and performed actions to develop a system to review and improve the quality of a product or process.
Quality management	Designs the co-ordinated activities to direct and control an organisation with regard to quality.
Quality system	The organisational structure, 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 retrieved 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.

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, distributed tissues and cells suspected or known to be potentially harmful.
Recipient	Person to whom human tissues, cells or embryos are applied.
Registry	A repository of data collected on tissue, cell and organ donors and/or transplant recipients for the purpose of outcome assessment, quality assurance, healthcare organisation, research and surveillance.
Reproductive cells (oocyte, sperm)	All cells intended to be used for the purpose of assisted reproduction.
Retrieval or recovery	See 'Procurement'.
Return	Sending back tissues or cells that may or may not present a quality or safety defect to the tissue establishment that supplied them for clinical 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 identifying the factors that resulted in the nature, magnitude, location and timing of a harmful or potentially harmful outcome.

Secondary packaging	Any material employed in the packaging of tissues and cells, excluding any outer packaging used for transportation or shipment not intended to be in direct contact with the graft.
Serious adverse event	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 patient or which might result in, or prolong, hospitalisation or morbidity.
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, incapacitating or which results in, or prolongs, hospitalisation or morbidity.
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. Both layers are separated by a thin sheet of fibres called 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.
Standard operating procedure	Written instructions describing the steps in a specific process, including the materials and methods to be used and the expected result.

Sterile	Free from biological contaminants.
Sterilisation	Any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spores, etc.) present on a surface, contained 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.
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.
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	Sperm-preparation technique based on the ability of sperm 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	When the method applied results in a sterility assurance level of 10–6.
Testicular sperm extraction	Extraction of testicular sperm(s) by biopsing the testes through one or multiple small incisions.

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 a tissue establishment on the basis of a contact or written agreement.
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 body where the activities of processing, preservation, storage or distribution of human tissues and cells are undertaken. It may also be responsible for procurement or testing of tissues and cells.
Toxicity	Degree to which a substance can damage a living or non-living organism.
Traceability	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. This implies the ability to identify the donor and the tissue establishment or the processing facility that receives processes or stores the tissue and cells, and the ability to identify 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 recipient originating from the tissues or cells applied.
Transplantation/ implantation/ grafting	Transfer (engraftment) of human tissues or cells from a donor to a recipient with the aim of restoring function(s) in the body.
Transport	Means used to transfer or convey tissues and cells from one place to another.
Trophectoderm	Outer layer of cells in a blastocyst (composed of trophectoderm and inner cell mass cells).
Unique identification code (donor number)	A code that unambiguously identifies a particular donor or donation.
Validation	Establishing documented evidence that provides a high degree of assurance that a specific process, piece of equipment or environment will consistently produce a product meeting its pre-determined specifications and quality attributes. A process is validated to evaluate the performance of a system with regard to its effectiveness, based on intended use.
Vasa deferentia	Tubes that transport sperm from the epididymis to the ejaculatory ducts.
Vigilance	An alertness or awareness of serious adverse events, serious adverse reactions or complications related to donation and clinical application of tissues, cells and organs involving an established process at a local, regional, national or international level for reporting.

Vitrification	Ultra-rapid cryopreservation method that prevents ice formation within the suspension, which is converted to a 'glass-like' solid.
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, which subsequently divides to form an embryo.

Appendix 4

Sample consent form

NHS form FRM 4281/3.2

FORM FRM4281/3.2 Effective: 12/05/14

Consent in accordance with the Human Tissue Act (2004)

NHS Blood and Transplant

Unique Tissue Number K | | | | | | | | | ODT Donor number | | | | | | |

Consent - Solid Organ and Tissue Donation

Directions for Completion:

1. This form must be completed in accordance with the Human Tissue Act 2004.
2. This six page form should be completed by the Specialist Nurse-Organ Donation (SN-OD)/ Nurse Practitioner – Tissue Services in **black or dark blue ink**, and signed and dated as appropriate.
3. Where an option box requires completion, it **must** be initialled by the SN-OD/Nurse Practitioner – Tissue Services/ Tissue Donor Co-ordinator.
4. The original should be retained by the SN-OD/ Nurse Practitioner – Tissue Services/ Tissue Donor Co-ordinator for the donor file.
5. A copy should be made for the patient's medical records.
6. Another copy should be offered to the patient's family.
7. A copy should be sent to relevent tissue banks.

NOTE: The term 'patient' is used throughout the form to refer to the potential donor.

FORM FRM4281/3.2 Effective: 12/05/14

NHS Blood and Transplant

Consent in accordance with the Human Tissue Act (2004)

Unique Tissue Number K | | | | | | | | |

ODT Donor number | | | | | | |

PATIENT DETAILS **Section 1**

Name

Address

Postcode

NHS number | | | | | | | | | | |

Hospital number | | | | | | | | | | | |

Date of birth | | | | | | | | |

Age (*If under 3 years record years and months*) | | years | | | months

CONSENT FOR ORGANS AND TISSUE **Section 2**

Complete **Box A** if the patient is/has giving/given first person consent
OR
Complete **Box B** if consent is given by the person ranking highest in the qualifying relationship

Box A

Name of patient

gave/gives* first person consent for the donation of the following organs/tissue for transplantation via the Organ Donor Register/donor card/expressed wish/will*
*(*Delete as appropriate)*

Box B

I, (*Name*) the (*Relationship to the patient*) of (*Patient's name*)

give my consent for the donation of organs and tissue as detailed below.

NOTE:
Proceed to Section 3 for Telephone Consent for Organ and/or Tissue Donation
Proceed to Section 4 for Organ and Tissue Donation
Proceed to Section 5 for Tissue Only Donation

OBTAINING CONSENT VIA TELEPHONE **Section 3**

To obtain consent via telephone is in accordance with the Human Tissue Act 2004 and the Human Tissue Authority Codes of Practice 2009 – it is not a legal requirement for relatives to sign a consent form. However, the interviewer **must** ask the following and **initial** the appropriate boxes:

	Yes	No
Do you agree to the conversation about donation between (name of HCP) of NHS Blood and Transplant and you being voice recorded? The recording will be stored as proof of the information that I give to you and of the consent and information that you give to me.		
For the purpose of the recording can you tell me again your full name and relationship to (name of the patient)		
May we use the recording and case details for training purposes?		

NOTE:
Proceed to Section 4 for Organ and Tissue Donation
Proceed to Section 5 for Tissue Only Donation

FORM FRM4281/3.2 Effective: 12/05/14

NHS Blood and Transplant

Consent in accordance with the Human Tissue Act (2004)

Unique Tissue Number K | | | | | | | | | ODT Donor number | | | | | | |

CONSENT FOR ORGAN DONATION — Section 4

Organ Group	Yes	No
All Abdominal Organs	☐	☐
All Cardiothoracic Organs	☐	☐

Organ	Yes	No	Coroner Objection	Outside Criteria
Kidneys	☐	☐	☐	☐
Liver	☐	☐	☐	☐
Liver hepatocytes	☐	☐	☐	☐
Pancreas	☐	☐	☐	☐
Pancreas Islet cells	☐	☐	☐	☐
Bowel	☐	☐	☐	☐
Other (consider multivisceral donation where appropriate) If YES	Please specify			
Heart	☐	☐	☐	☐
Lungs	☐	☐	☐	☐
Blood Vessels	☐			

CONSENT FOR TISSUE DONATION — Section 5

All Tissue	Yes	No	Coroner Objection
	☐	☐	☐

Tissue	Yes	No	Coroner Objection	Outside Criteria
Eyes	☐	☐	☐	☐
Whole heart for heart valve donation	☐	☐	☐	☐
Skin	☐	☐	☐	☐
Bone	☐	☐	☐	☐
Tendons	☐	☐	☐	☐
Meniscus	☐	☐	☐	☐
Spleen (tissue only donation)	☐	☐	☐	☐

Specific Tissue	Yes	No	Coroner Objection	Outside Criteria
Corneas	☐	☐	☐	☐
Sclera	☐	☐	☐	☐
Whole heart for heart valve donation	☐	☐	☐	☐
Pericardium	☐	☐	☐	☐
Femur(s)	☐	☐	☐	☐
Iliac crest(s)	☐	☐	☐	☐
Achilles tendons	☐	☐	☐	☐
Semi tendinosus tendons	☐	☐	☐	☐
Patella tendons	☐	☐	☐	☐
Anterior tibialis tendons	☐	☐	☐	☐
Meniscus	☐	☐	☐	☐

Blood Vessels	☐	☐	☐	☐	Please specify
Other	☐	☐	☐	☐	Please specify

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FORM FRM4281/3.2 Effective: 12/05/14

NHS Blood and Transplant

Consent in accordance with the Human Tissue Act (2004)

Unique Tissue Number K | | | | | | | | | ODT Donor number | | | | | | |

REQUIRED INFORMATION TO SUPPORT ORGAN AND TISSUE DONATION **Section 6**

IF APPLICABLE (Organ and Tissue Donation)

Do you consent to the patient being transferred from their place of death to the dedicated donation facility for the donation procedure to be undertaken? **Yes** ☐ **No** ☐

I have been informed of and am aware of the following:

That blood and tissue samples will be obtained from the patient (and the patient's mother where the patient is under 18 months old **and/or** the patient has been breast fed in the last 12 months) for testing, including tissue typing, HIV, Hepatitis, HTLV and Syphilis. These samples may be subsequently stored for future testing as necessary. In the event of any screening results that may have implications for the family, relevant individuals may be contacted if their health could be affected.

That blood and tissue samples e.g lymph node and spleen that have been obtained for screening will be subsequently biopsied, analysed and stored for future testing as necessary.

Blood vessels will be retrieved and stored to support surgical procedures and if not used within 14 days will be disposed of in accordance with the hospital/tissue establishment policy. The tissue donated for transplantation will be stored for extended periods in tissue establishments whilst it is prepared for transplantation.

The patient's medical records will be accessed by relevant healthcare professionals to obtain a past medical/behavioural history. This information may be passed on a need-to-know basis to other healthcare professionals in support of the transplantation process. This information may also be retained by the Organ Donation Teams/Tissue Establishments.

Organs and / or tissue which you have agreed to donate may be used in research prior to transplantation to improve transplant outcomes for the recipient. The organs and / or tissue will still be transplanted. Do you consent to this? **Yes** ☐ **No** ☐

CONSENT FOR SCHEDULED PURPOSES **Section 7**

Organs and/or tissue can also be used for the scheduled purposes listed below.

Scheduled Purposes Include:
Research
Education or Training related to Human Health
Clinical audit
Quality Assurance
Performance Assessment

Organs and/or tissue may at times be found to be unsuitable for transplantation once removed.
Do you consent to organs and/or tissue being used for a scheduled purpose listed above if they are removed for transplant and subsequently found unsuitable? **Yes** ☐ **No** ☐

Other organs and/or tissue unsuitable for transplantation may also be donated to aid future health care.
Do you consent to organs and/or tissue being removed solely for a scheduled purpose as listed above (please detail in notes on page 6)? **Yes** ☐ **No** ☐

NOTE:
Organs and/or tissue will be used and stored for a scheduled purpose in accordance with The Human Tissue Act (2004)

Organs and/or tissue that are not used for a scheduled purpose, or following the completion of a scheduled purpose, will be disposed of as per local establishment policy in accordance with the Human Tissue Act (2004).

FORM FRM4281/3.2 Effective: 12/05/14

NHS Blood and Transplant

Consent in accordance with the Human Tissue Act (2004)

Unique Tissue Number K | ODT Donor number

CONFIRMATION OF CONSENT **Section 8**

I have read and understood the above and I have had the opportunity to ask and have had my questions answered

Patient/Relationship to patient

Name (Please print) Signed

Date 2 0 Time *(24 hr)* at :

Address of person giving consent

Telephone number

Mobile

Co-signatory Name *(Where applicable)* (Please print) Signed

Healthcare Professional Details (Witness)

Designation

Name (Please print) Signed

Date 2 0 Time *(24 hr)* at :

Specialist Nurse – Organ Donation/Nurse Practitioner – Tissue Services/ Tissue Donor Co-ordinator

Designation

Name (Please print) Signed

Date 2 0 Time *(24 hr)* at :

	Yes	No
Did the patient give first person consent for the donation via the Organ Donor Register/donor card/expressed wish/will?		
Did the patient have a nominated representative?		
Was consent obtained from the person ranking highest in the qualifying relationship?		

Persons are ranked in the following descending order:
- **A)** Spouse or partner (including civil or same sex partner)
- **B)** Parent or child
- **C)** Brother or sister
- **D)** Grandparent or grandchild
- **E)** Niece or nephew
- **F)** Stepfather or stepmother
- **G)** Half-brother or half-sister
- **H)** Friend of longstanding

If no, please give details below:

Information leaflets given to family? OR Information leaflets to be sent to the family?

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Consent in accordance with the Human Tissue Act (2004)

NHS Blood and Transplant

Unique Tissue Number K | | | | | | | | | ODT Donor number | | | | | | |

NOTES

Appendix 5

Sample donor assessment form

Extracted from NHS form 4211/1.1 and donor assessment rationale (form PA1 (V03))

NHS
Blood and Transplant

Patient Assessment Form

Directions for completion

1 This six-page form must be completed in **black or dark blue ink** by the SN-OD/NP/ANP/Tissue Transplant Co-ordinator and signed where required.

2 The original copy should be retained by the **SN-OD/NP/ANP/Tissue Co-ordinator** for the donor file.

3 A copy should be made for the patient's medical records.

4 In the event of organ and tissue donation, a legible photocopy should be sent to the relevant **Tissue Establishment/CTS Eye Bank**, where required.

NOTE: The term patient is used throughout the form to refer to the potential donor.

NHS
Blood and Transplant

ODT Donor number

PATIENT ASSESSMENT *(Information obtained from relative/significant other)*

Patient's name (Please print)

Donor hospital

Hospital/CHI number

Cause of death:

Patient date of birth *(dd/mm/yyyy)*

In order to proceed with organ and tissue donation it is necessary for us to ask you some questions - which should be answered to the best of your knowledge - about your relative's medical and behavioural history. All information will be treated with the strictest confidence.

For paediatric patients under the age of 18 months or those who have been breast-fed in the past twelve months the mother is required to answer these questions with regard to both her own and her child's health.

For children: has your child been breast-fed in the last twelve months? Yes ☐ No ☐ Not applicable ☐

NOTE: For all donors under the age of 18 months and any baby or child who has been breastfed in the last 12 months, a blood sample is required from the mother of the donor

For ALL female patients aged between 13 and 53 years of age

Is there a possibility that your relative could be pregnant? Yes ☐ No ☐ Unknown ☐

GENERAL HEALTH INFORMATION

1 Has your relative visited his/her general practitioner in the last two years? Was he/she currently seeing or waiting to see their general practitioner or any other healthcare professional? Yes ☐ No ☐ Unknown ☐

If YES, give details

2 Did your relative have diabetes? Yes ☐ No ☐ Unknown ☐

If yes, were they on insulin? Yes ☐ No ☐ Unknown ☐

Is there a family history of diabetes? Yes ☐ No ☐ Unknown ☐

If yes, is it insulin dependent diabetes? Yes ☐ No ☐ Unknown ☐

3 Did your relative take regular medication? Yes ☐ No ☐ Unknown ☐

If YES, give details

4 Did your relative ever undergo any investigations for cancer or have they ever been diagnosed with cancer? Yes ☐ No ☐ Unknown ☐

If YES, give details

5 Did your relative recently suffer from any significant weight loss? Yes ☐ No ☐ Unknown ☐

If YES, give details

6 Did your relative have any signs of recent infection, eg colds, flu, fever, night sweats, swollen glands, diarrhoea, vomiting or skin rash? Yes ☐ No ☐ Unknown ☐

If YES, please specify

7 Did your relative come into contact with any infectious disease recently or have any immunisations within the last eight weeks? Yes ☐ No ☐ Unknown ☐

If YES, give details

8 Did your relative ever have hepatitis, jaundice or liver disease? Yes ☐ No ☐ Unknown ☐

If YES, give age and any diagnosis

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Patient Assessment Form

ODT Donor number

GENERAL HEALTH INFORMATION continued

9 Did your relative have a history of ocular disease or previous eye surgery or corrective laser treatment? Yes ☐ No ☐ Unknown ☐

If YES, give details

10 Did your relative ever suffer from any bone, joint, skin or heart disease, eg rheumatic fever? Yes ☐ No ☐ Unknown ☐

If YES, give details

11 Did your relative ever have any operations or illnesses including an organ or tissue transplant? ***If no go to question 13.*** Yes ☐ No ☐ Unknown ☐

If YES, give details

12 Did your relative ever have neurosurgical operations for tumour or cyst of the spine/brain or implantation of dura mater, before August 1992? Yes ☐ No ☐ Unknown ☐

If YES, please specify

13 Did your relative receive a blood, or blood product/component transfusion (such as Fresh Frozen Plasma (FFP), Platelet, Cryoprecipitate or Immunoglobulins) at any time (particularly since1980)? Yes ☐ No ☐ Unknown ☐

If YES, give details

14 Was your relative ever told **never** to donate blood? Yes ☐ No ☐ Unknown ☐

If YES, give details of where, when and the reason

15 Did your relative suffer from any chronic or autoimmune illness or disease of unknown cause, eg inflammatory bowel disease, multiple sclerosis, sarcoidosis? Yes ☐ No ☐ Unknown ☐

If YES, give details

16 Did your relative suffer from any type of brain disease such as Parkinson's disease, Alzheimer's disease, recent memory loss, confusion or unsteady gait? Yes ☐ No ☐ Unknown ☐

If YES, give details

17 Did your relative have a family history of prion disease or do you know if they were ever told that they were at risk of CJD, vCJD, GSS or FFI? Yes ☐ No ☐ Unknown ☐

If YES, give details

18 Did your relative ever receive human pituitary extracts, eg growth hormones, fertility treatment or test injections for hormone imbalance? Yes ☐ No ☐ Unknown ☐

If YES, give details

19 Did your relative ever have any other serious infection such as tuberculosis, malaria, West Nile virus, SARS, typhoid fever, toxoplasmosis, rabies, encephalitis, Lyme disease or brucellosis? Yes ☐ No ☐ Unknown ☐

If YES, give details, and any treatment received

20 Did your relative have any acupuncture, tattooing, body piercing, botox, injections or cosmetic treatments that involves piercing the skin in the last six months? Yes ☐ No ☐ Unknown ☐

If YES, give details

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GENERAL HEALTH INFORMATION continued

21 In the last twelve months has your relative been in close contact with a bat anywhere in the world or been bitten by an animal whilst abroad? Yes ☐ No ☐ Unknown ☐

If YES, give details of animal and place of treatment

22 Did your relative ever have a sexually transmitted infection e.g. syphilis, gonorrhoea, genital herpes, genital warts? Yes ☐ No ☐ Unknown ☐

If YES, give details of diseases, dates and treatment

TRAVEL RISK ASSESSMENT

23 Did your relative ever travel outside the UK?
*(If NO or UNKNOWN, proceed to **26b** then continue with Behavioural Risk Assessment. If YES, continue with questions below)* Yes ☐ No ☐ Unknown ☐

24 Has your relative travelled outside the UK in the last 12 months? Yes ☐ No ☐ Unknown ☐

If yes, give details of date of visit/return and destination.

25 Ever had a fever or received treatment for an illness whilst abroad or within six months of leaving an area where there is malaria or West Nile Virus? Yes ☐ No ☐ Unknown ☐

If YES, give date of fever/illness and places visited

26 (a) Ever live or work in rural Central or South America for a continuous period of four weeks or more? Yes ☐ No ☐ Unknown ☐

If YES, specify place and date of last visit, and details of living conditions

26 (b) Was your relative or their mother born in Central or South America? Yes ☐ No ☐ Unknown ☐

(c) Was your relative ever given a blood transfusion in that country? Yes ☐ No ☐ Unknown ☐

27 (a) Ever spend a continuous period of six months or longer in an area where there is malaria at any time during his/her life? Yes ☐ No ☐ Unknown ☐

(b) If YES, have they travelled to a malaria area since then? Yes ☐ No ☐ Unknown ☐

If YES, give details of where

BEHAVIOURAL RISK ASSESSMENT

28 Did your relative:

(a) consume alcohol? Yes ☐ No ☐ Unknown ☐

If YES, approximately how many units per week

(b) smoke tobacco or any other substance? Yes ☐ No ☐ Unknown ☐

If YES, give details

29 Is it possible that any of the following apply to your relative?

(a) is, or may be infected with HTLV, HIV or hepatitis B or C? Yes ☐ No ☐ Unknown ☐

(b) has ever injected or been injected with non-prescriptive drugs, including body building drugs, even if it was a long time ago or only once? Yes ☐ No ☐ Unknown ☐

(c) has ever been given payment for sex with money or drugs? Yes ☐ No ☐ Unknown ☐

(d) *(for male patients only)* ever had sex with another man with or without a condom? Yes ☐ No ☐ Unknown ☐

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BEHAVIOURAL RISK ASSESSMENT continued

(e) *(for female patients only)* had sex in the last 12 months with a man who has had sex with another man with or without a condom?	Yes ☐	No ☐	Unknown ☐
(f) been in prison or a juvenile detention centre for more than three consecutive days within the last 12 months? **NB: This excludes those who have been in a police cell for <96 hours.**	Yes ☐	No ☐	Unknown ☐
(g) had sex in the last 12 months with:			
(i) anyone who is HIV or HTLV positive?	Yes ☐	No ☐	Unknown ☐
(ii) anyone who has hepatitis B or C?	Yes ☐	No ☐	Unknown ☐
(iii) anyone who had a sexually transmitted disease?	Yes ☐	No ☐	Unknown ☐
(iv) anyone who has ever been given payment for sex with money or drugs?	Yes ☐	No ☐	Unknown ☐
(v) anyone who has ever injected drugs?	Yes ☐	No ☐	Unknown ☐
(vi) anyone who may ever have had sex in any part of the world where AIDS/HIV is very common (this includes most countries in Africa)?	Yes ☐	No ☐	Unknown ☐

Having answered all the previous questions is there anyone else who you think may provide more information? Yes ☐ No ☐

If YES, please specify

Question number	Relevant additional information

Information discussed with

Name (Please print) | Relationship (Please print)

Signature of healthcare professional obtaining information | Please print name

Designation of healthcare professional obtaining information

Date of interview / / 20 | Time of interview

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Rationale document for patient assessment form PA1 (v03)

Introduction

The purpose of patient assessment is firstly to determine if a potential donor is suitable to donate *any* organ or tissue and then to determine *which* organs and tissues can be donated. Whilst the donor may 'in general' be acceptable for donation, not all organs or tissues may be suitable due to 'system specific' medical problems. This document aims to provide a rationale for specific information that is required to assess a potential donor's suitability for organ/tissue donation and should be used in conjunction with the NHS Blood and Transplant **FRM4211** Patient Assessment Form (PA1).

The purpose of risk assessment is to determine risk factors for the transmission of disease from donor to recipient. It is the responsibility of the Specialist Nurse – Organ Donation (ODT), Nurse Practitioner/ Assistant Nurse Practitioner (Tissue Services) and Tissue Transplant Co-ordinator (SNBTS to collect comprehensive information on medical, behavioural and travel history and relay all of the information obtained to the organ recipient and tissue procurement centres. In addition, for organs, it is the responsibility of the implanting surgeon to assess the risk of transplant for their individual patients. For tissues, it is the responsibility of the tissue establishment to make the final decision on donor suitability.

Risk is relative to the risks of not receiving a transplant.

The Specialist Nurse – Organ Donation (ODT), Nurse Practitioner/ Assistant Nurse Practitioner (Tissue Services) and Tissue Transplant Co-ordinator (SNBTS) must be familiar with the purpose of each question and must recognise when to expand the question in order to obtain more details and what additional information might be required. The conditions which will cause the deferral of a potential donation vary significantly between organs, ocular tissue and other tissues. For potential tissue donors further detailed information regarding the deferral criteria for each type of tissue can be found in the

current version of the UKBTS Tissue Donor Selection Guidelines for Deceased Donors (TDSG-DD). Due to the avascular nature of corneal grafts, many of the deferral criteria for other tissues do not apply to cornea.

For all paediatric donors under the age of 18 months, and any infant donor over the age of 18 months but who has been breast-fed in the past 12 months, the mother is required to answer the questions in the patient assessment document with regard to both her own and her child's health.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
For paediatric donation: has your child been breast-fed in the last 12 months?	There is a risk of vertical transmission of some viral infections from the mother to her child via breast milk. This may be determined by the mother's medical and behavioural history that is used as a surrogate for the infant's history. If yes, the medical history of the mother will need to be assessed and a maternal blood sample must be taken for virology testing.	Not an absolute contraindication; inform recipient centres and ensure the following sampling takes place: • All babies and children who have been breastfed within 12 months of donation should have maternal sampling. • Neonates (less than 2 months) – maternal only sampling. • Babies greater than 2 months not breastfed should have samples from the infant with maternal samples as a fallback position if required. • Babies greater than 18 months not breastfed should only require infant sampling.	Provided the mother's blood sample is found to be negative for markers of viral infection, this is not a contraindication to donation. Ensure the following sampling takes place: • All babies and children who have been breastfed within 12 months of donation should have maternal sampling. • Neonates (less than 2 months) – maternal only sampling. • Babies greater than 2 months not breastfed should have samples from the infant with maternal samples as a fallback position if required. • Babies greater than 18 months not breastfed should only require infant sampling.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
For all female patients between 13 and 53 years of age Is there a possibility that your relative could be pregnant?	If there is a possibility that the patient could be pregnant then a pregnancy test should be performed, to determine whether the foetus is viable. This would have a direct effect upon whether donation is able to proceed or not.	If the foetus is not determined to be viable there is no contraindication to donation.	Donation acceptable.
General health information			
Did your relative/significant other:			
1. Visit his/her general practitioner in the last 2 years? Was he/she currently seeing or waiting to see their general practitioner or any other healthcare professional?	1.1. These are broad questions to quickly ascertain if the donor has on-going health problems. If the answer to either is yes, it is important to obtain as much information as possible.	Donation acceptable.	A positive answer is not itself a contraindication to donation: however each condition must be assessed for its acceptability as per the current version of TDSG-DD.
	1.2. Note: It is important to obtain accurate information on past medical history. Therefore it is a requirement that the GP be contacted to complete the NHSBT GP questionnaire (FRM1602).	For organ donation this should be done pre-donation. Attempts to should always be made to contact the GP before retrieval of organs. If these attempts do not enable contact with the GP this **must** be completed within 3 working days.	For tissue only donation this is usually done post-donation.
2. Have diabetes? If yes, were they on insulin?	Due to the effect diabetes can have on a number of organs particularly the kidneys additional tests/information relating to function may be necessary.	Not an absolute contraindication except for pancreas; inform recipient centres.	Donation acceptable except for pancreatic tissue.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
3. Take regular medication?	3.1. Very few drugs are themselves contraindications to donation but knowledge of the donor's drug therapies may indicate an underlying disease that is itself a contraindication to donation for some tissues. It is useful to know why the medication was being taken although doses and frequency are not required.	Not an absolute contraindication; inform recipient centres.	Certain drugs may exclude the donation of specific tissues, e.g. long-term steroid therapy may affect the quality of bone and skin. See TDSG-DD and seek advice from tissue establishment.
	3.2. A small number of drugs may cause birth defects in babies exposed to them while in the womb (consider potentially pregnant organ recipients). It is important to allow time for the drugs to be cleared from the donor. It takes longer to clear some drugs than others.	Not an absolute contraindication; inform recipient centres.	For tissue donation other than cornea; isoretinoin (roaccutane), acitretinate (neotigason), etretinate (tigason) used to treat acne and dutasteride (avodart) and finasteride (proscar) used to treat prostatic hyperplasia all have specified deferral periods. See current version of TDSG-DD.
	3.3. Individuals being treated with immunosuppressive drug therapy, such as transplant recipients may not be eligible to donate; as the serology test may be misleading In addition any infection maybe masked.	Not an absolute contraindication; inform recipient centres.	Must not donate if immunosuppressed.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
4. Ever undergo any investigations for cancer or ever been diagnosed with cancer?	4.1. The presence, or previous history, of cancer poses a risk of transmission of cancer cells to a recipient. If yes, obtain further information regarding dates and treatments.	Not an absolute contraindication; inform recipient centres. It is important to assess the type and grade of cancer. Please refer to Council of Europe document (1997) for more information.	Depending on the type cancer can very often be acceptable for corneal tissue donation but usually not for other tissues. See current version of TDSG-DD.
5. Recently suffer from any significant weight loss?	5.1. Recent weight loss may be an indication of illness, which includes cancer. It is important therefore to obtain the reason for the weight loss.	Not an absolute contraindication; inform recipient centres.	Not an absolute contraindication – depends on underlying cause.
6. Have any signs of recent infection, e.g. colds, flu, fevers, night sweats, swollen glands, diarrhoea, vomiting and skin rash?	6.1. Bacterial, viral and protozoal infections can all be transmitted by transplantation. Successful antibiotic treatment may make donation acceptable.	Not an absolute contraindication; inform recipient centres. However it is important to ascertain specific information about the organism so that appropriate antibiotic/antifungal treatment may be administered to the recipient.	Active systemic infection is a contraindication to most tissue donation but cornea donation may be possible. Localised infection may be acceptable. Each condition must be assessed for its acceptability as per the current version of TDSG-DD.
	6.2 Many of these symptoms can also be signs of underlying malignancy.	Not an absolute contraindication; inform recipient centres.	Each condition must be assessed for its acceptability as per the current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
7. Come into contact with any infectious diseases recently or have any immunisations within the last 8 weeks?	7.1. Potential donors who have been in recent contact with an infectious disease (for which they have no history of previous infection) may be in the asymptomatic stage of developing an infection at the time of donation.	Not an absolute contraindication; inform recipient centres.	See current version of TDSG-DD.
	7.2 Immunisations with live vaccine may cause severe illness in people who are immunosuppressed. By 8 weeks any infection caused by the immunisation should have been controlled and so should not be passed on through donated material. There are special rules for BCG and smallpox immunisations.	Not an absolute contraindication; inform recipient centres.	Unacceptable for tissue donation if less than 8 weeks since receiving a live immunisation.
8. Ever have hepatitis, jaundice or liver disease?	8.1. Viral hepatitis is readily transmitted by all types of transplantation. Any history of jaundice or hepatitis must therefore be investigated. Testing alone may not exclude all infectious donors and the donor history may suggest the need for additional testing. However, jaundice can be caused by many non-infectious conditions, e.g. gallstones, obstruction of the bile ducts, congenital biliary atresia or neonatal jaundice.	Not an absolute contraindication; inform recipient centres.	See current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
9. Have a history of ocular disease or previous eye surgery or corrective laser treatment?	9.1 This question is specifically designed to assess the suitability of ocular tissue.	Not an absolute contraindication; inform recipient centres.	Corneal disease and infections, e.g. herpes, ocular inflammation, retinoblastoma and malignant tumours of the anterior segment are contraindications to eye donation. Laser refractive surgery (e.g. LASIK) to the cornea is also a contraindication. However, other existing eye disease or previous eye surgery does not necessarily exclude corneas from transplantation. See current version of TDSG- DD or where appropriate seek specific specialist advice.
10. Ever suffer from any bone, joint, skin or heart disease, e.g. rheumatic fever?	10.1. This question relates to the suitability of specific tissues. Whilst the donor may 'in general' be acceptable for donation not all tissues may be suitable due to 'system specific' medical problems. This question is aimed at identifying some of these medical diseases.	Inform recipient centres of details of specific diseases. Inform recipient centres of details of specific diseases.	The presence of disease in any of these systems may preclude donation of that specific tissue. See current version of TDSG-DD. See current version of TDSG-DD.
	10.2 Note however that some tissue specific symptoms may be part of a systemic disease, e.g. SLE, and therefore a general deferral for the donation of tissues.	Inform recipient centres of details of specific diseases.	See current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
11. Ever have any operations or illnesses, including an organ or tissue transplant?	11.1. The first part of this question is to quickly ascertain if the donor has had previous significant health problems. If the answer is yes, it is important to obtain as much information as possible. Surgery may be related to underlying malignancy.	Not an absolute contraindication; inform recipient centres.	Each condition must be assessed for its acceptability as per the current version of TDSG-DD.
	11.2. The question regarding transplantation is a SaBTO requirement. There is the risk of viral or prion transmission when someone has received a tissue transplant. There has been one definite and one probable case of CJD transmission by corneal transplants.	Individual assessment is required.	If dura mater or ocular tissue was transplanted no tissue donations can be accepted. A history of receipt of other tissue transplants since 1980 is a contraindication for most types of tissue donation, with the exception of skin or heart valve donation in some circumstances refer to TDSG-DD and seek advice from tissue establishment.
	11.3. There is the risk of viral or prion transmission when someone has received an organ transplant. Individuals being treated with immunosuppressive drug therapy, such as transplant recipients may not be eligible to donate, as the serology test may be misleading. In addition any infection may be masked.	Individual assessment is required.	A history of receipt of an organ is a contraindication for all types of tissue donation.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
12. Ever have neurosurgical operations for a tumour or cyst of the spine/brain or implantation of dura mater, before August 1992?	12.1. This is to ascertain if the donor may have been given a dura mater graft as part of a neurosurgical procedure. This material is known to have transmitted CJD in around 200 cases. Brain surgery often required dura mater repair. Neurosurgeons may use different materials for this but before 1992; dura mater from cadaveric donors was used in brain and spinal surgery. Spinal fusion and burr holes did not usually involve using dura mater.	Not an absolute contraindication; inform recipient centres.	If yes, tissue donation can only be accepted if it can be shown that dura mater was not used.
13. Receive a blood transfusion/blood product/component transfusion (such as Fresh Frozen Plasma (FFP), Platelet, Cryoprecipitate or Immunoglobulins) in the last 12 months or at any other time (particularly since 1980)?	13.1. Blood or blood product/component transfusion (such as Fresh Frozen Plasma (FFP), Platelet, Cryoprecipitate or Immunoglobulins) transfusions have transmitted bacterial, viral, protozoan and prion infections. Testing of blood donors for markers of infection varies by country and also by date. A complex set of criteria exist for tissue donor acceptability depending on when and where the transfusion took place and also for the type of tissue to be donated. To date there have been 4 cases of vCJD and 2 cases of asymptomatic prion transmission by blood transfusion. The question regarding transfusion is a SaBTO requirement. See TDSG-DD for detailed guidance.	Not an absolute contraindication; inform recipient centres.	See current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
	13.2. If there has been **significant blood loss and replacement of fluids** with blood components and/or colloids within the 48-hour period prior to obtaining the donor's blood sample there may be significant haemodilution of the sample. This may result in 'false negative' results when testing the donor for viral markers of infection. If no pre-transfusion sample is available, a detailed assessment of all intravenous fluid intakes during the 48-hour period before sampling is required to enable the haemodilution calculation to be performed.	Not an absolute contraindication; inform recipient centres.	If there is > 50% haemodilution no tissue donations can be accepted.
	13.3. The reason for the blood transfusion should be obtained as this may itself be a contraindication to donation.	Inform recipient centres of details of specific diseases.	See current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
14. Ever told never to donate blood?	14.1. Must establish reason why person told never to give blood. There are a number of individuals who have been informed that they are classified as being at "increased risk" of CJD/vCJD for public health purposes because they have been exposed to possible risk through blood transfusion, surgery, or tissue transplantation. The individuals have all been informed that they should not donate blood, tissues or organs. Examples are: • Individuals who had surgery using instruments that had been used on someone who developed CJD. • Individuals who have given blood to someone who later developed vCJD. • Individuals who have received more than 80 units of blood or blood components.	Not a contraindication to donation; inform recipient centres.	Contraindication if person told never to give blood owing to CJD risk.
	14.2. Individuals may have been told not to donate for other reasons, for example HCV infection or for a haematological disorder.	Not a contraindication to donation; inform recipient centres.	See current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
15. Suffer from any autoimmune illnesses or disease of unknown aetiology, e.g. inflammatory bowel disease, multiple sclerosis and sarcoidosis?	15.1. Some diseases of unknown aetiology may have an infectious origin and may be transmissible.	Not an absolute contraindication; inform recipient centres.	Acceptance criteria are specific for each condition, see TDSG-DD. For example multiple sclerosis is an absolute contraindication for all tissues whilst for sarcoidosis ocular tissue can be donated provided there is no actual ocular involvement.
	15.2. Inflammatory bowel disease can also increase the risk of bacteria entering the blood stream.	Not an absolute contraindication; inform recipient centres.	Crohn's disease and ulcerative colitis are exclusions for tissue donation except for corneal donation.
	15.3 Autoimmune disease is caused by the body attacking itself and can be either limited to a single organ, e.g. thyroid disease or affect multiple systems, e.g. rheumatoid disease. Severe systemic disease may adversely affect the quality of a number of tissues. In addition treatment to suppress the condition may be with steroids, immunosuppressive drugs, anti-metabolites or antibodies directed against part of the immune system. This may well make the donor more susceptible to certain types of infection and also make some infections more difficult to diagnose.	Not an absolute contraindication; inform recipient centres.	Acceptance criteria are specific for each condition, see TDSG-DD. For example multiple sclerosis is an absolute contraindication for all tissues whilst for sarcoidosis ocular tissue can be donated provided there is no actual ocular involvement.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
16. Suffer from any type of brain disease such as Parkinson's disease, Alzheimer's disease, motor neurone disease, recent memory loss, confusion or unsteady gait?	16.1. CNS disease may be: • of suspected infective origin, e.g. multiple sclerosis or CJD, or • a neurodegenerative condition of unknown aetiology, e.g. Parkinson's disease or Alzheimer's disease. There are concerns that Alzheimer's disease may mask symptoms of CJD. In the event of confusion or memory loss the risk of CJD has to be excluded.	Not an absolute contraindication; inform recipient centres.	Contraindication unless confusion and/or memory loss has an underlying clinical reason that is itself not a contraindication to transplantation.
17. Have a family history of CJD, vCJD, Gerstmann-Straussler-Schienker disease, or Fatal Familial Insomnia?	17.1. These are all varieties of prion-associated disease. 10-15% of classical CJD cases are associated with gene mutations (familial CJD). Individuals at familial risk of prion-associated disease are those who have 2 or more blood relatives with a prion-associated disease or where the family has been informed it is at risk following genetic testing and counselling.	Contraindication.	Contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
18. Ever receive human pituitary extracts, e.g. growth hormones, fertility treatment or test injections for hormone imbalance?	18.1. Prior to 1985 treatment with growth hormone, infertility treatment and some thyroid diagnostic tests used material derived from the pituitary glands of untested cadavers, some of whom may have died from CJD. Around 200 recipients of this material subsequently developed CJD.	Not an absolute contraindication; inform recipient centres.	Contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
19. Ever have any other serious infection, e.g. tuberculosis, malaria, West Nile virus, SARS, typhoid fever, toxoplasmosis, rabies, encephalitis, Lyme disease or brucellosis?	19.1. This question picks up a range of infections which could be transmitted by transplantation. It is important to obtain as much information as possible to determine the degree of risk of transmission to a recipient. Please refer to the Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO) (February, 2011) for further information. West Nile virus, which has been identified as a disease that is a potential risk to organ and tissue recipients, must be identified where possible. However, where some patients are asymptomatic, relevant travel risks must also been noted (Question 25). Note: West Nile virus is active during certain seasons, and migrates across the world; therefore healthcare professionals must remain vigilant as to areas of recent outbreaks.	Not an absolute contraindication; inform recipient centres. *Active Tuberculosis* Or treatment within 6 months is not an absolute contraindication to donation. If the donor has still to complete a course of chemotherapy the recipient patient will require appropriate drug treatment. A previous history of tuberculosis is not a contraindication to donation other than at the original site of infection, e.g. liver. *Malaria* Contraindication to donation if there is a known active infection and no curative chemotherapy has been given. *West Nile virus* Contraindication to donation if there is a known active infection. Incubation is up to 14 days; therefore relevant travel history is a requirement.	Each condition must be assessed for its acceptability as per the current version of TDSG-DD. In all cases active infection is a contraindication to donation. There are a variety of deferral periods relating to either the date of cessation of symptoms or the date of termination of treatment. Some infections are a permanent contraindication to donation, whilst for malaria it is also dependant on the results of antibody testing.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
20. Have any acupuncture, tattooing, body piercing, Botox injections or cosmetic treatment that involves piercing the skin in the last 6 months?	20.1. This question aims to identify donors who may be at risk of having been exposed to reused needles. Acupuncture, tattooing, body piercing, Botox injections or cosmetic treatment that involves piercing the skin all carry a low risk to transmit viral disease. Most tattooist and piercers work to high standards, using disposable needles, but not all do. In the UK there have been occasional large outbreaks of both HBV and HCV as a result of poor hygienic standards. None of these activities are reasons to reject a donor if they were carried out more than 6 months prior to donation. It is helpful, if possible, to know where and when the treatment was carried out.	Not an absolute contraindication; inform recipient centres.	For tissue donors the deferral period has been reduced from 12 months to 6 months since all tissue donors are tested for anti-HBc.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
21. In the last 12 months either been in close contact with a bat anywhere in the world or bitten by any mammal outside the UK?	21.1. Animal bites may result in many different infections. This question aims to identify donors who may be at risk of having been exposed to rabies. There have been 2 recent cases where organ donors transmitted rabies to all recipients of their organs and to the recipient of a blood vessel. Historically there have been a small number of cases of rabies transmitted by corneal transplantation. In the UK the only risk of rabies comes from contact with infected bats whilst outside the UK bites from infected mammals, especially dogs, are also major routes of infection.	Contraindication.	Contraindication – see TDSG-DD. In addition, bites from a non-human primate at any time are a **permanent** contraindication to tissue donation.
22. Ever have a sexually transmitted infection, e.g. syphilis, gonorrhoea, genital herpes, genital warts?	22.1. A history of sexually transmitted infection is often not immediately forthcoming from relatives when enquiring about someone's general health. This question is to specifically raise this topic in isolation to evoke either a positive or negative response. If the answer to it is yes, it is important to obtain as much information as possible. Untreated STDs can eventually cause damage to many organs and tissues.	Not an absolute contraindication; inform recipient centres.	Acceptance criteria are specific for each condition, see TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
Travel risk assessment			
This group of questions is designed to establish the risk of donated organs and tissues transmitting a number of serious infections which are not found within the UK. Due to the forever changing pattern of infections worldwide, when a history of travel abroad has been obtained it is necessary to consult both the TDSG-DD and the Geographical Disease Risk Index (GDRI) for up-to-date information on the specific deferral criteria current at the time. These can be obtained by accessing the JPAC (Joint Professional Advisory Committee) website www.transfusionguidelines.org.uk which is updated as information becomes available. (If access to this website is not available to the SN-OD the NHSBT Duty Office will access this for you.)			
Did your relative/significant other:			
23. Ever travel outside the UK?	23.1. This opening question, if negative for travel, allows rapid progression to the next set of questions without the need to answer further travel questions. If the answer is yes, it is important to obtain as much information as possible based on the subsequent questions.	Not an absolute contraindication; inform recipient centres.	Refer to the TDSG-DD and GDRI.
24. Travel outside the UK in the last 12 months? If yes, please give details of date of visit/return and destination.	24.1. Twelve months is referred to as this is the longest temporary deferral period for tropical infections. Other infections (both tropical and non-tropical) have shorter deferral periods. Corneal tissue is treated differently from other tissues as it is avascular not considered to be a risk of transmitting protozoal infections such as malaria or Trypanosoma cruzi infection.	Not an absolute contraindication; inform recipient centres.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue, 'visitors' to a malarial area < 6 months ago are not acceptable, 6-12 months ago require a malaria antibody test, > 12 months ago are acceptable. Refer to the TDSG-DD and GDRI.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
25. Ever have a fever or treatment for an illness whilst abroad or within 6 months of leaving an area where there is malaria or West Nile virus?	25.1. Malaria and other endemic infections such as West Nile virus can be transmitted by blood, viable organs, tissues and cells therefore it is important to determine the nature of the illness. Note: A malaria antibody test is of no use if taken prior to 6 month incubation period.	*Malaria* Not an absolute contraindication; inform recipient centres. **Ensure blood sample for malaria screen is sent to the appropriate reference laboratory for all high risk patients.** *West Nile virus* Contraindication.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue a malaria antibody test is required. Refer to the TDSG-DD and GDRI.
26a Ever live or work in rural Central or South America for a continuous period of 4 weeks or more?	26a.1. Individuals who have ever lived in Central or South America are at risk of Trypanosoma cruzi infection, which is caused by a parasite transmitted by an insect vector, which bites humans and animals at night time. Those at most risk are trekkers, backpackers and soldiers on manoeuvres in jungle areas as they may have been living in primitive areas and/or sleeping out in the jungle.	Not an absolute contraindication; inform recipient centres.	For corneal tissue T. cruzi is not a deferral criterion. For other tissues a T. cruzi antibody test is required. Refer to the TDSG-DD and GDRI.
26b. Was the deceased or their mother born in Central or South America?	26b.1. T. cruzi infection can be passed vertically from mother to child so that a child born outside this area and who has never travelled to this area is still at risk of infection.	Not an absolute contraindication; inform recipient centres.	For corneal tissue T. cruzi is not a deferral criterion. For other tissues a T. cruzi antibody test is required. Refer to the TDSG-DD and GDRI.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
26c. Given a blood transfusion in that country?	26c.1. As T. cruzi is endemic in this area and individuals remain asymptomatic for years after infection many blood donors are infected by this organism. T. cruzi is readily transmitted by blood transfusion from an infected donor. Screening and treatment of blood in this area is becoming more widespread but is still not universal. See also Question 13.	Not an absolute contraindication; inform recipient centres.	For corneal tissue T. cruzi is not a deferral criterion. For other tissues a T. cruzi antibody test is required. Refer to the TDSG-DD and GDRI.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
27a. Ever stay for 6 months or longer in an area where there is malaria, at any time in his/her life?	27a.1. This question is designed to make it possible to establish whether a potential donor meets the required criteria of malaria area 'resident'. Individuals who have lived in a malaria affected area for more than 3 months before the age of 5 years develop a partial immunity to malaria through repeated exposure. Partial immunity means that people may be infected with the malaria parasite but show no symptoms, sometimes for years. These individuals were classified as 'residents' as opposed to 'visitors' and, as they pose a much higher risk of transmitting infection, were managed in a different way to people who had simply visited a malaria area. More recently the definition of 'resident' was extended to include all individuals who have resided in a malaria area for a continuous period of 6 months at any time in their lives. Note: A malaria antibody test is of no use if taken prior to 6 month incubation period.	Not an absolute contraindication; inform recipient centres. **Ensure a blood sample for malaria screen is sent to the appropriate reference laboratory for all high risk patients.**	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue a malaria antibody test is required. Refer to the TDSG-DD and GDRI.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
27b. If yes, ever travelled outside the UK since then?	27b.1. An individual who is classified as 'resident' is managed differently from a non-resident for each subsequent visit to a malaria area no matter how short the visit. A malaria antibody test is required for all non-corneal tissue even if it > 12 months since the last visit.	Not an absolute contraindication; inform recipient centres. Ensure a blood sample for malaria screen is sent to NHSBT Colindale for all high risk patients.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue a malaria antibody test is required. Refer to the TDSG-DD and GDRI.
Behavioural risk assessment **To the best of your knowledge did your relative:**			
28a. Consume alcohol?	28a.1. The effect of alcohol can impact on the quality of liver tissue. If yes, it is important to obtain as much information as possible.	Not an absolute contraindication; inform recipient centres.	Not a contraindication.
28b. Smoke tobacco or other substances?	28b.1. The effect of smoking can impact on the quality of lung tissue. If yes, it is important to obtain as much information as possible.	Not an absolute contraindication; inform recipient centres.	Not a contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
Behavioural risk assessment			
Based on information obtained from blood donors who tested positive and epidemiological data from larger populations, it is known that certain groups of people may be at increased risk of infection by HIV, HCV and HBV. Unfortunately it is not possible to exclude all cases of infection by relying on blood testing alone as infected donors may be missed in the very early stages of infection, commonly referred to as the 'window period'. This refers to the period between being infected and the appropriate test being able to detect the infection. It takes around 10-12 days to start to form antibodies and a number of weeks before the antibody levels are high enough to be detected by a test that is based on antibody detection. Tests that are based on antigen detection will pick up the infection earlier but it still takes 10-20 days (depending on the specific virus) for adequate numbers of viral particles to be present in the blood to be detected. During all this period the potential 'negative' donor is highly infectious and any organ or tissue transplant will transmit the infection. For this reason donors found to be in any of the known high risk groups must be excluded from tissue donation on the basis of history alone.			
To the best of your knowledge, is it possible any of the following applies to your relative:			
29a. Is or may be infected with HTLV, HIV or hepatitis B or C?	29a.1. HIV/hepatitis B or C can be transmitted via organ/tissue donation therefore it is vital to identify anyone who is known to be, or thinks that they may be infected with the viruses.	HIV disease is an absolute contraindication, however HIV infection is not. Hepatitis B or C are not absolute contraindications: inform recipient centres.	Contraindication.
29b Ever injected or been injected with non-prescribed drugs, including body-building drugs, even if it was a long time ago or only once?	29b.1. People with a history of intravenous drug use remain the largest group with HCV infection in the UK. They also have a higher rate of HIV and HBV infection. It is important to obtain as much information as possible to assess possible risk behaviour. Viral infection can be transmitted by sharing equipment used to inject drugs.	Not an absolute contraindication; inform recipient centres.	Contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
29c. Ever received payment for sex with money or drugs?	29c.1 People who receive payment for sex have a higher risk of contracting HIV/hepatitis B or C and other sexually transmitted diseases due to the high number of sexual partners and the promiscuity of these partners. In addition this group of people often sell sex to fund a drug habit. This further increases the risk of infection within this group.	Not an absolute contraindication; inform recipient centres.	Contraindication.
29d. (for male patients only) Ever had sex with another man, with or without a condom?	29d.1. Men who have sex with men have a much higher prevalence of HIV infection and this activity remains the leading cause of HIV infection within the UK.	Not an absolute contraindication; inform recipient centres.	Contraindication.
29e. (for female patients only) Had sex in the last 12 months with a man who has ever had sex with another man, with or without a condom?	29e.1. As these infections can be transmitted sexually; there is also a higher risk of infection for the sexual partners of individuals who fall into any of these above categories. A temporary deferral for 12 months from the time of the last exposure is used to prevent the risk of any 'window period' infections from being transmitted.	Not an absolute contraindication; inform recipient centres.	Contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
29f. Has been in prison or a juvenile detention centre for more than 3 consecutive days within the last 12 months? Note: This excludes those who have been in a police cell for < 96 hours.	29f.1. It is known that there is a higher risk for individuals who are in prison of being exposed to transmissible viruses through sexual contact and intravenous drug abuse. For a living donor these questions would be asked directly but for a deceased donor this is not possible. It is felt that relatives, who sometimes do not even normally reside with the donor, are unlikely to be able to answer these questions especially relating to the period in prison. As it is essential to rely on virology testing only, the possibility of a 'window period' infection must be excluded by use of a deferral period. It is therefore important to identify individuals who have been exposed to this environment.	Not an absolute contraindication; inform recipient centres.	Contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
29g. Had sex in the last 12 months with: i. Anyone who is HIV or HTLV positive? ii. Anyone who has hepatitis B or C? iii. Anyone who had a sexually transmitted disease? iv. Anyone who has ever had payment for sex with money or drugs? v. Anyone who has ever injected drugs? vi. Anyone who may ever have had sex in a part of the world where AIDS/HIV is very common (this includes most countries in Africa)?	29g.1. There is a higher risk of contracting HIV through heterosexual intercourse in some parts of the world where the virus is endemic. It is therefore important to identify individuals who fall within this category. As these infections can be transmitted sexually, there is also a higher risk of infection for the sexual partners of individuals who fall within the above categories. A temporary deferral for 12 months from the time of the last exposure is used to prevent the risk of any 'window period' infections from being transmitted.	Not an absolute contraindication; inform recipient centres.	Contraindication.
Having answered all the previous questions is there anyone else who you think may provide more information?		*This question provides the opportunity to suggest others who may have alternative knowledge of any aspects of the patient's history. For example parents for past medical history or close friends for behavioural history.*	

Appendix 6

Sample tissue donor physical assessment form

AATB Guidance Document No. 1, v2. Tissue Donor Physical Assessment Form (27 June 2005)

Identification

Name stated on Consent (Authorization) : _____

Age: _____ ☐days ☐months ☐years Recovery Agency ID#: _____

Sex/gender: ☐Male ☐Female Race: _____ _____ID#:_____

Weight: _____ ☐lbs. ☐kgs Weight is: ☐estimated/team ☐reported (source: _____) ☐actual

Height: _____☐ft. ☐in. ☐cm. Height is: ☐estimated/team ☐reported (source: _____) ☐actual

Manner identified by: ☐hospital ID band ☐toe tag ☐other (describe) _____

Identification Band/Tag

ID re-created as closely as possible,

or check here ☐ if N/A (not present)

Personnel confirming donor identification: _____Date/time:_____

Evidence of Donation/Autopsy

Eye donor: ☐whole eyes ☐corneas only ☐N/A ; Organ donor: ☐Yes ☐No UNOS#: _____

Autopsy: tissue recovery is ☐pre or ☐post autopsy (☐full ☐limited); ☐no autopsy planned;

or, ☐plan for autopsy unknown

Recovery Team Assessment:

Is there evidence of:

Jaundice ——— ☐Yes ——— ☐No

Genital lesions ——— ☐Yes ——— ☐No

Enlarged lymph nodes ——— ☐Yes ——— ☐No

Tattoo/piercing ——— ☐Yes ——— ☐No

White spots in the mouth ——— ☐Yes ——— ☐No ——— ☐Unable to visualize

Non-medical injection sites ——— ☐Yes ——— ☐No

Enlarged liver (hepatomegaly) ——— ☐Yes ——— ☐No

Insertion trauma/perianal lesions ——— ☐Yes ——— ☐No

Rash/scab/skin lesion (non-genital) ——— ☐Yes ——— ☐No

Blue/purple (gray/black) spots/lesions ——— ☐Yes ——— ☐No

Trauma/infection to potential retrieval sites ——— ☐Yes ——— ☐No

Abnormal ocular finding (e.g., icterus, scarring)—— ☐Yes ——— ☐No ——— ☐Unable to visualize

Notes/Explain if “unable to visualize”, or if any answers are “Yes”: _____

General Appearance

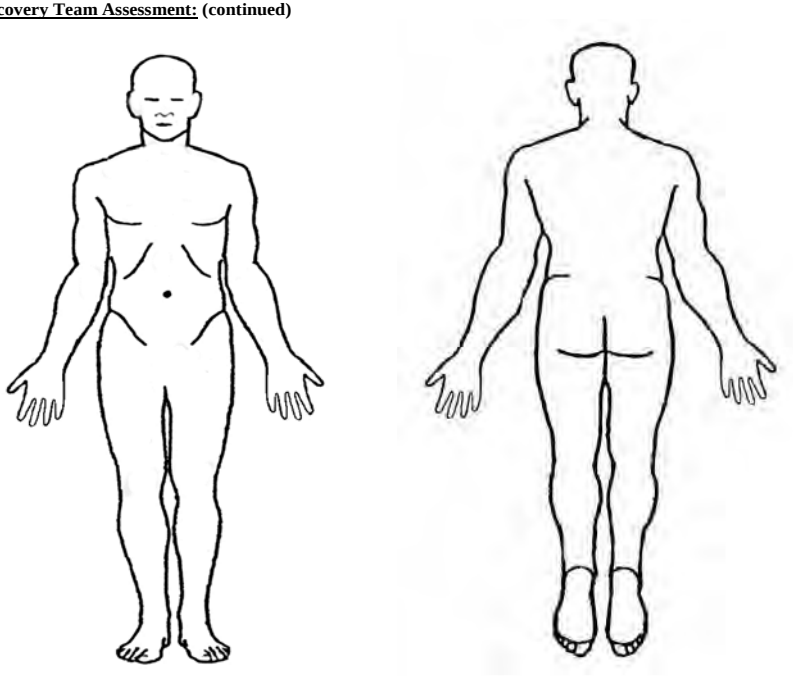
Cleanliness: ☐Good ☐Poor; Describe if “poor” _____

Personnel performing physical assessment: _____Date/time:_____

_____	_____	_____	_____
Name of Person Completing Form (Print)	Signature	Initials	Date

Sample Tissue Donor Physical Assessment Form
Recovery Agency ID#: _____

Recovery Team Assessment: (continued)



Key to schematics:

(A) Abrasion	(J) Team blood draw site	(T) Tattoo – requires description
(B) Bruise/Contusion	(L) Laceration/Wound	(U) Urethral catheter
(C) Cast/Ortho device	(M) ID band/tag	(V) Skin lesion
(D) Dressing/Bandage	(N) Needle entry site	(W) Scab
(E) ET tube/NG tube	(O) Organ recovery incision	( ) _____
(F) Fracture/Dislocation	(P) Body Piercing – requires description	( ) _____
(H) Hematoma	(R) Rash	
(I) IV/Arterial line	(S) Scar (surgical/trauma)	

Summary
A review of available medical records & physical assessment findings were completed & found to be acceptable/not acceptable prior to recovery. _____ _____

(Circle one) (Responsible person) (Date/time)

Appendix 7

Sample haemodilution algorithm

APPENDIX 7: SAMPLE HAEMODILUTION ALGORITHM

Donor ID # _____

Date/Time of sampling _____ a.m./p.m.

Donor's weight _____ kg

Blood Volume (BV) = donor's weight (kg) _____ ÷ 0.015

or

(BV) = donor's weight (kg) _____ × 70 mL/kg _____ mL (**BV**)

Plasma Volume (PV) = donor's weight (kg) _____ ÷ 0.025

or

(PV) = donor's weight (kg) _____ × 40 mL/kg _____ mL (**PV**)

A. Total volume of **Blood** transfused (last 48 hours before death or sample collection, whichever comes first)

Volume of: Red Blood Cells transfused/48 hours _____

+ whole blood transfused/48 hours _____ **A** = _____mL

B. Total volume of **Colloid** administered (48 hours before death or sample collection, whichever comes first)

Volume of: dextran _____ mL

+ plasma _____ mL

+ platelets _____ mL

+ albumin _____ mL

+ hetastarch _____ mL

+ other _____ mL **B** = _____ mL

C. Total volume of **Crystalloid** infused (within 1 hour before death or sample collection, whichever comes first)

Volume of: saline _____ mL

+ Dextrose in water _____ mL

+ Ringer's lactate _____mL

+ other _____mL **C** = _____ mL

Determination of Sample Acceptability for Infectious Disease Testing: Calculate both 1 and 2

1. Is **B** + **C** > **PV**? Yes No

2. Is **A** + **B** + **C** > **BV**? Yes No

* 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; either use a pre-transfusion/infusion sample or other qualified sample or the donor is not eligible.

Reference: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm091345.pdf.

Appendix 8

Validation of screening for infectious disease assays for use with blood from deceased donors

The reliability of the results of screening for infectious diseases on blood samples from deceased individuals is critical and can be enhanced substantially if appropriately validated assays are used. Assays for infectious diseases specifically labelled for use to screen blood donors are considered suitable to use for 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, 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 a possibility for a false-negative test result. Although sensitivity and specificity are important for any screening assay, whether samples from living or deceased individuals are tested, sensitivity is the most important 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 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;

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;

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 cutoff and vary 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. 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.

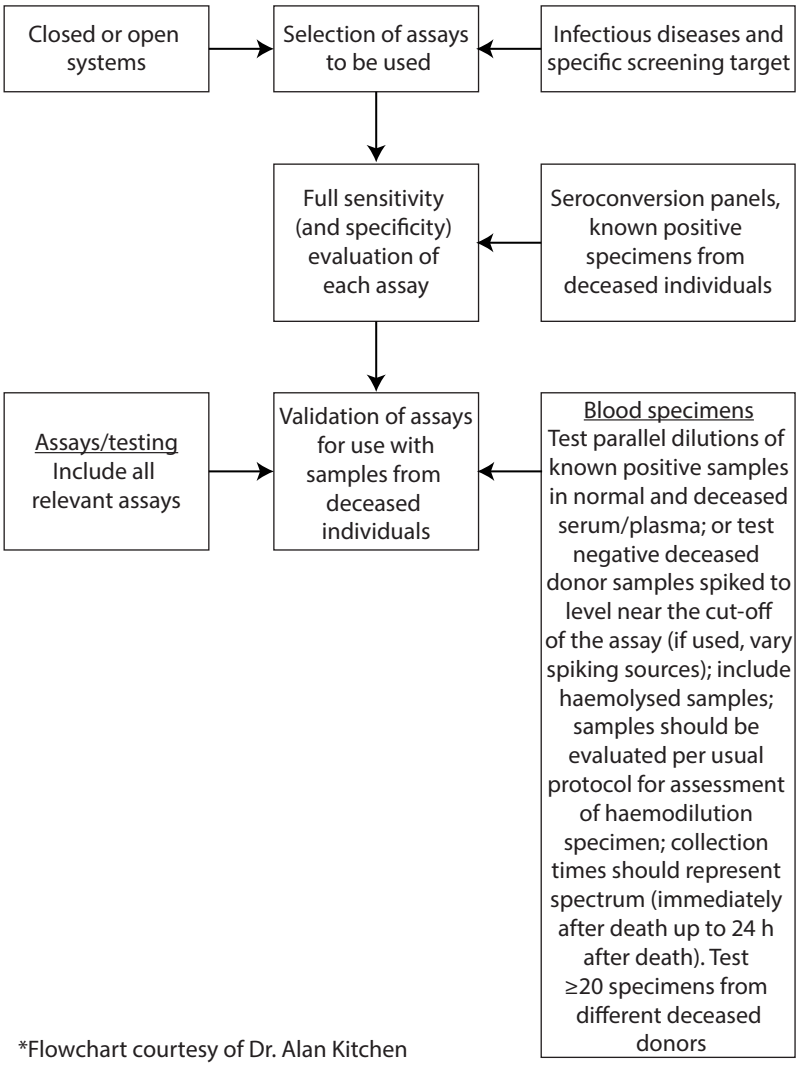
Nonetheless, before using assays with blood specimens from deceased individuals, the performance of the assays must be assessed in some way to make attempts to demonstrate there is no loss of, or other change in, the expected performance of the assay.

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 due to 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 due to 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 for any markers of a previously unidentified and relevant infection to 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, etc.). 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.

Validation Scheme*



*Flowchart courtesy of Dr. Alan Kitchen

Appendix 9

Sample form to assess the suitability of the working environment

Extracted from NHS FRM3831/5.1

FORM FRM3831/5.1 Effective: 16/12/13

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:**

(Template Version 07/10/08)

Cross-Referenced in Primary Document: MPD557 Page 1 of 12

Appendix 10

Sample donor identification form

Extracted from NHS FRM3831/5.1

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

(Template Version 07/10/08)

Appendix 11

Example of a process validation

Tissue transportation[1]

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 provided below refers specifically to the transportation 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 was achieved by addressing the following questions:

- Which type of tissue and what maximum volume will be transported?

1 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.

- 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?
- Which refrigerant has been used and what is its specification and volume?
- What are the specifications of the transport container (i.e. dimensions, insulation, etc.)?
- What are the most extreme transportation conditions allowable in terms of transport time and ambient temperature?

Once the process had been defined, the acceptance criteria needed to be defined. In our example, they were that the:

- Temperature of the skin allograft must remain at 0–10 °C for the duration of the transit.
- Integrity of the tissue packaging must be maintained during transit.
- Integrity of the transport container must be maintained during transit.
- 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).

It was determined that the maximum amount of skin that would 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 transportation 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.

Note, however, 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 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 confirm that they have complied with the relevant standard operating procedures.

Appendix 12

Adverse reaction or event impact assessment tool

The Impact Assessment tool assists practitioners and regulators in planning their response to a given adverse reaction or event taking into account broad 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/ 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 a Health Authority to a specific serious adverse reaction or event (SARE) should be proportionate to the potential impact as assessed by the matrix described above.

White The tissue establishment to manage the corrective and preventive actions and the Health Authority to file the report and keep a 'watching brief'.

Pale shading Requires interaction between the tissue establishment and the Health Authority, which may request an inspection that focuses on the SARE and 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 shading 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 as done previously 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:

- reducing the probability of recurrence through preventive measures;
- increasing the detectability of the risk; or
- reducing the severity of the consequences, if it should recur.

Appendix 13

Summary of data reported in the 2011 EU-wide report of serious adverse reactions and serious adverse events associated with the clinical application of tissues and cells (data reported for 2010)

Extracts from the report[1]

Serious Adverse Reactions

A total of 460 serious adverse reactions (SAR) were reported for 2010 by 14 EU member states and Croatia (which was not an EU member state at that time). According to the data, when compared with the total amount of tissues and cells distributed, SAR occurred only in 0.14% of cases. However, this percentage should be interpreted with caution because only 20 countries provided data for the distribution of tissues and cells and, in some cases, it was acknowledged that data submitted were only partial due to difficulties in collection of information from some of the end users (hospitals/clinics).

1 The full report compiled by the European Commission from information submitted by member states and EEA countries is available at http://ec.europa.eu/health/blood_tissues_organs/docs/tissues_cells_adverse_events_2011_en.pdf.

Fifteen countries reported that no SAR related to the human application of human tissues and cells was reported in their countries in 2010. At this stage it is not possible to make any comparison between countries because, in some cases, a high number of reported SAR indicates that a reliable and accurate reporting system is in place whereas, in other cases, the absence/low number of reported SAR cannot always be interpreted as an impeccably functional system.

Of the 460 SARs reported:

- 291 SARs were related to the human application of reproductive cells and tissues (gametes),
- 108 SARs were related to transplantations of haematopoietic stem cells (including bone marrow, blood peripheral stem cells, cord blood, donor lymphocyte infusions and other stem cells)
- 52 SARs were related to transplantation of replacement tissues (bone, tendon and ligaments, cornea, heart valves, amniotic membrane)
- 9 SARs were related to therapies with advanced therapy medicinal products (ATMP), which included human cells.

No SARs were reported for the following categories of tissues and cells: cartilage and other skeletal tissues, skin, sclera and other ocular tissues, blood vessels and other cardiovascular tissues, chondrocytes, hepatocytes, pancreatic islets, other tissues (e.g. fat tissues, umbilical cord segments), embryos and other reproductive tissues (e.g. ovarian or testicular tissues).

Serious Adverse Events

Fifteen member states as well as Lichtenstein and Croatia provided data regarding the number of tissues and cells processed in 2010. For the purpose of this reporting, the term 'tissues and cells processed' refers to tissues and cells processed in the tissue establishments, but not necessarily distributed to the end users. Overall, 477 039 units of tissues and cells were reported to be processed in 2010. This is likely to be an underestimation at this time.

Serious adverse events (SAE) were reported by 17 member states and Norway. The total number of SAE reported for 2010 was 451, representing 0.095% of the tissues and cells processed in the same period of time. As in case of SAR, the percentage of SAE in relation to the total number of tissues and cells processed should be interpreted with care because some of the countries reporting SAE did not provide the number of tissues and cells processed at the national level; in some cases it was mentioned that reported data were not provided for all categories of tissues and cells processed in 2010.

Of the 451 reported SAE:

- 74 SAEs (16.41%) were linked to 'Procurement';
- 26 SAEs (5.76%) were linked to 'Testing';
- 16 SAEs (3.55%) were linked to 'Transport';
- 172 SAEs (38.14%) were related to 'Processing';
- 34 SAEs (7.54%) were linked to 'Storage';
- 40 SAEs (8.86%) were linked to 'Distribution';
- 33 SAEs (7.32%) were linked to 'Materials';
- 56 SAEs (12.42%) were included in the category 'Other SAE'.

The 451 SAE were attributed to one of four specifications:

- Tissue and cell defects: 149 SAE (33.04%);
- Human error: 168 SAEs (37.25%);
- Equipment failure: 81 SAE (17.96%);
- Other: 53 SAEs (11.75%).

The completeness and accuracy of these EU reports will improve over time as individual member states develop their systems for gathering data in compliance with the definitions included in this questionnaire.

Appendix 14

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Human tissues and cells are being used in an increasing variety of ways, and advances in transplantation therapy have unquestionable benefits. Human cells and tissues for human application can save lives or restore essential functions, but the use of human tissues and cells also raises questions of safety and quality. Only tissues and cells recovered, processed and stored following strict quality and safety standards are likely to function satisfactorily. Careful evaluation of donors is essential to minimise the risk of transmission of diseases. Furthermore, since human tissues and cells can currently only be derived from the body of a person, strong ethical principles need to be associated with their donation and use.

The Council of Europe approaches the donation and human application of human tissues and cells in compliance with the principles of non-commercialisation and voluntary donation of materials of human origin. This 2nd Edition of the *Guide to the quality and safety of tissues and cells for human application* contains information and guidance for all professionals involved in identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood collection centres, tissue establishments processing and storing tissues and cells (including assisted reproductive technology centres), inspectors auditing any of these establishments and organisations responsible for human application.

For matters dealing with the use of organs and blood or blood products, see the Council of Europe *Guide to the quality and safety of organs for transplantation* and *Guide to the preparation, use and quality assurance of blood components*, respectively.

ENG

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