Radiation Sterilization of Tissue Allografts:

Requirements for Validation and Routine Control

A Code of Practice

Sample size (n)	SAL ($1/n$)	Bioburden															
		0.65	0.73	0.83	0.93	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.6	3. 0	3.2	4.0	4.4
10	1/10	1.0	1.1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.7	1.8	1.9	2.0	2.1	2.2	2.3
15	1/15	1.3	1.3	1.4	1.5	1.5	1.7	1.8	1.9	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6
20	1/20	1.4	1.5	1.6	1.7	1.7	1.9	2.0	2.1	2.2	2.2	2.3	2.4	2.5	2.6	2.8	2.9
25	1/25	1.6	1.7	1.7	1. 8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	3.0	3.0
30	1/30	1.7	1.8	1.9	2.0	2.0	2.1	2.3	2.4	2.5	2.5	2.6	2.7	2.9	2.9	3.1	3.2
35	1/35	1.8	1.9	2.0	2.1	2.1	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.3	3.4
40	1/40	1.9	2.0	2.1	2.2	2.2	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5
45	1/45	2.0	2.1	2.2	2.3	2.3	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.5	3.6
50	1/50	2.1	2.1	2.2	2.3	2.4	2.5	2.7	2.8	2.9	3.0	3.0	3.2	3.3	3.4	3.6	3.7
60	1/60	2.2	2.3	2.4	2.5	2.5	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5	3.5	3.8	3.9
70	1/70	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.2	3.3	3.3	3.5	3.6	3.7	3.9	4.0
80	1/80	2.4	2.5	2.6	2.7	2.8	2.9	3.1	3.2	3.3	3.4	3.5	3.6	3.8	3.8	4.0	4.1
90	1/90	2.5	2.6	2.7	2.8	2.9	3.0	3.2	3.3	3.4	3.5	3.6	3.7	3.9	3.9	4.1	4.2
100	1/100	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5	3.6	3.7	3.8	4. 0	4.0	4.2	4.3

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RADIATION STERILIZATION OF TISSUE ALLOGRAFTS: REQUIREMENTS FOR VALIDATION AND ROUTINE CONTROL

A CODE OF PRACTICE

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Sales and Promotion, Publishing Section
International Atomic Energy Agency
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fax: +43 1 2600 29302
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FOREWORD

These recommendations for the radiation sterilization of tissue allografts adopt the principles that the International Organization for Standardization (ISO) applies to the radiation sterilization of health care products. The approach has been adapted to take into account the special features associated with human tissues and the features that distinguish them from industrially produced sterile health care products.

The approach as described here is not applicable if viral contamination is identified. Thus it is emphasized that the human donors of the tissues must be medically and serologically screened. To further support this screening it is recommended that autopsy reports be reviewed if available. This adaptation of established ISO methods can thus only be applied to sterilization of tissue allografts if the radiation sterilization described here is the terminal stage of a careful, detailed, documented sequence of procedures involving: donor selection; tissue retrieval; tissue banking general procedures; specific processing procedures; labelling; and distribution.

The methods proposed here for the establishment of a sterilization dose are based on statistical approaches used for the sterilization of health care products and modified appropriately for the low numbers of tissue allograft samples typically available.

This code of practice will be useful to tissue banking staff, surgeons using tissues for transplantation, regulators who oversee the safety of transplantation and radiation sterilization procedures, members of tissue banking associations, health service personnel in hospitals in which tissue transplantations are performed and inter-governmental organizations involved in transplantation issues, for example the World Health Organization.

This publication was discussed extensively at an international meeting in Wrexham in the United Kingdom and was approved by the Technical Advisory Committee of the relevant IAEA project, which included the Chairpersons of the American Association of Tissue Banks, the Asia–Pacific Surgical Tissue Banking Association, the European Association of Tissue Banks and the Latin American Association of Tissue Banking. Paticular gratitude is expressed to G. Phillips and B. Parsons from the United Kingdom for their significant contributions to the development of this code of practice. The IAEA officer responsible for this publication was J. Hendry of the Division of Human Health.

EDITORIAL NOTE

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CONTENTS

1. INTRODUCTION

International standards have been established for the radiation sterilization of health care products, which include medical devices, medicinal products (pharmaceuticals and biological agents) and in vitro diagnostic products [1–8]. Following intensive studies of the effects of ionizing radiation on the chemical, physical and biological properties of tissue allografts and their components, these are now radiation sterilized using a variety of methods and practices.

Through its radiation and tissue banking project, the IAEA has established this code of practice for the radiation sterilization of tissue allografts and its requirements for validation and routine control of the sterilization of tissues.

Annex I describes the methods for selecting a sterilization dose. Annex II provides three worked examples applying these methods. There are also tables in Annex III that show microbial survival data relating to a standard distribution of resistance (SDR). A list of key references for the sterilization of tissues by ionizing radiation is included in the Bibliography.

This code of practice sets out the requirements of a process for ensuring that the radiation sterilization of tissues produces standardized sterile tissue allografts suitable for safe clinical use. Although the principles adopted in this code of practice are similar to those used for the sterilization of health care products, there are substantial differences in practice arising from the physical and biological characteristics of tissues.

For health care products, the items for sterilization come usually from large production batches; for example, syringes are uniform in size and have bacterial contamination arising from the production process, usually at low levels. It is the reduction of the microbial bioburden to acceptably low levels that is the purpose of the sterilization process, where such levels are defined by the sterility assurance level (SAL). The inactivation of microorganisms by physical and chemical means follows an exponential law, and so the probability of a microorganism surviving can be calculated if the number and type of microorganisms are known and if the lethality of the sterilization process is also known. Two methods are used to establish the radiation doses required to achieve low SAL values:

(a) Method 1 relies on knowing the bioburden (assuming an SDR) before irradiation and uses these data to establish a verification dose, which will indicate the dose needed for a SAL of 10^{-2}. The method involves a statistical approach to setting the dose based on three batches, and hence

relatively large numbers of samples are required for establishing both the initial bioburden and the verification dose, both per product batch. A further adaptation of method 1 for a single production batch has also been developed.

(b) In method 2, the bioburden levels are measured after giving a series of incremental doses to the samples, these doses being well below the dose required for a SAL of 10^{-6}, which is a generally acceptable level. In this method, 280 samples are required to determine the dose to produce a SAL value of 10^{-2}, from which the dose needed to yield a SAL value of 10^{-6} may be extrapolated. No assumptions are made in method 2 about the distribution of microorganisms and their resistances.

Method 1 has also been adapted to allow the use of as few as ten samples to determine the verification dose. In this modification, the dose needed for a SAL value of 10^{-1} is used to establish the dose required for a SAL value of 10^{-6}. The sole purpose of this modification, however, is to substantiate whether the conventionally used dose of 25 kGy is an appropriate dose to achieve a SAL value of 10^{-6}. Another method to substantiate the sterilization dose of 25 kGy was also developed.

2. STERILIZATION OF TISSUE ALLOGRAFTS

Tissues used as allografts comprise a wide range of materials and bioburden levels such that the above quality assurance methods developed for health care products cannot be applied without careful and due consideration given to the differences between health care products and tissue allografts.

Tissues that are sterilized currently include bone, cartilage, ligaments, tendons, fascias, dura mater, heart valves, vessels, skin and amnion. The variability in types and levels of bioburden in tissues is much greater than that found for health care products, where the levels of microbial contamination are usually low and relatively uniform in type and level.

In addition, tissue allografts are not products of commercial production processes involving large numbers of samples. These differences mean that extra attention must be given to the following:

(a) Uniformity of sample physical characteristics (shape and density);
(b) Uniformity of the bioburden in the sample;

(c) Donor screening for viral contamination;
(d) Whether low numbers of samples can be used for sterilization dose setting purposes.

The objective of this code of practice is to provide the necessary guidance in the use of ionizing radiation to sterilize tissue allografts in order to ensure their safe clinical use.

This code of practice specifies requirements for validation, process control and routine monitoring of the selection of donors, tissue processing, preservation and storage, and radiation sterilization of tissue allografts. The requirements apply to continuous and batch type gamma irradiators using the radioisotopes ^{60}Co and ^{137}Cs, electron beam accelerators and X rays.

The principles adopted here are similar to those described for health care products, in that statistical approaches to establishing doses to ensure sterility of the tissue products are proposed.

2.1. DEFINITIONS

The majority of the definitions relating to the sterilization process are given in Ref. [1]. The following definitions are particularly useful for this code of practice:

allograft. A graft transplanted between two different individuals of the same species.

allograft product. An allograft or a collection of allografts within a primary package.

absorbed dose. The quantity of radiation energy imparted per unit mass of matter. The unit of absorbed dose is the gray (Gy), where 1 gray is equivalent to the absorption of 1 joule per kilogram (1 Gy = 100 rad).

batch (irradiation). The quantity of final product irradiated at the same cycle in a qualified facility.

batch (production). The defined quantity of finished tissue product from a single donor that is intended to be uniform in character and quality, and which has been produced during a single cycle of processing.

bioburden. The population of viable microorganisms on the tissue allograft and package prior to the sterilization process.

distribution. The transport and delivery of tissues for storage or use in the recipient.

dose mapping. An exercise conducted within an irradiation facility to determine the distribution of the radiation dose throughout an amount of tissue allograft or simulated items of specified bulk density, arranged in irradiation containers in a defined configuration.

dosimeter. A device having a reproducible, measurable response to radiation, which can be used to measure the absorbed dose in a given material.

dosimetry system. A system used for determining absorbed dose, consisting of dosimeters, measuring instrumentation and procedures for the system's use.

D_{10}. The radiation dose required to inactivate 90% of the homogeneous microbial population, where it is assumed that the death of microbes follows first order kinetics.

good tissue banking practice (GTBP). A practice that meets accepted standards as defined by relevant government or professional organizations.

irradiator. An assembly that permits safe and reliable sterilization processing, including the source of radiation, conveyor and source mechanisms, safety devices and shield.

positive test of sterility. A test of sterility that exhibits undetectable microbial growth after incubation in a suitable culture medium.

qualification. Obtaining and documenting evidence concerning the processes and products involved in tissue donor selection, tissue retrieval, processing, preservation and radiation sterilization that will produce acceptable tissue allografts.

recovery efficiency. Measure of the ability of a specified technique to remove microorganisms from a tissue allograft.

reference standard dosimeter. A dosimeter of high metrological quality used as a standard to provide measurements traceable to, and consistent with, measurements made using primary standard dosimeters.

routine dosimeter. A dosimeter calibrated against a primary or reference dosimeter and used routinely to make dosimetric measurements.

sample item portion (SIP). A defined standardized portion of a tissue allograft that is tested.

sterile. Free of viable microorganisms.

sterility assurance level (SAL). The probability of a viable microorganism being present on a tissue allograft after sterilization.

sterilization. A validated process to destroy, inactivate or reduce micro-organisms to a sterility assurance level (SAL) of 10^{-6}. (Sterility is expressed in several national legislations and international standards as a SAL of 10^{-6}.)

sterilization dose. The minimum absorbed dose required to achieve the specified sterility assurance level (SAL).

test of sterility. A test performed to establish the presence or absence of viable microorganisms on a tissue allograft, or portions thereof, when subjected to defined culture conditions.

tissue bank. An entity that provides or engages in one or more services involving tissue from living or dead individuals for transplantation purposes. These services include assessing donor suitability, tissue recovery, and tissue processing, sterilization, storage, labelling and distribution.

validation. Refers to establishing documentary evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. A process is validated to evaluate the performance of a system with regard to its effectiveness based on intended use.

verification dose. The dose of radiation to which a tissue allograft, or portions thereof, is nominally exposed in the verification dose experiment with the intention of achieving a predetermined sterility assurance level (SAL).

2.2. PERSONNEL

Responsibility for the validation and routine control for sterilization by irradiation, including tissue donor selection and tissue retrieval, processing, preservation, sterilization and storage, shall be assigned to qualified personnel in accordance with subclauses 6.2.1 or 6.2.2 in Ref. [9], whichever is applicable.

3. VALIDATION OF PRESTERILIZATION PROCESSES

3.1. GENERAL

An essential step in the overall radiation sterilization of tissues is rigorous donor selection to eliminate specific contaminants. Full details about donor selection, tissue retrieval, tissue banking general procedures, specific processing procedures, labelling and distribution are given in the IAEA International Standards for Tissue Banks, which are being prepared. Such tissue donor selection and tissue retrieval, processing and preservation are processes that determine the characteristics of the tissue allograft prior to the radiation sterilization process. The most important characteristics are those relating to use of the tissues as allografts, namely their physical, chemical and biological properties, the latter including the levels and types of microbial contamination. Validation of these processes shall include the following:

(a) Qualification of tissue bank facilities;
(b) Qualification of tissue donors;
(c) Qualification of tissue processing and preservation;
(d) Certification procedure to review and approve documentation of (a–c);
(e) Maintenance of validation;
(f) Process specification.

3.2. QUALIFICATION OF TISSUE BANK FACILITIES

Tissue banks shall have facilities to receive procured tissues and to prepare tissue allograft material for sterilization. Such facilities are expected to include laboratories for the processing, preservation and storage of tissues prior to sterilization. These laboratories and the equipment contained therein

shall meet international standards enunciated by the various tissue bank professional associations.

A regular documented system should be established that demonstrates that these standards are maintained, with special emphasis on the minimization of contamination by microorganisms to bioburden levels throughout the tissue retrieval, transport, processing, preservation and storage stages.

Tissue banks shall also have access to qualified microbiological laboratories to measure the levels of microorganisms on the tissue allografts at various stages in their preparation for the purposes of assessing both the levels of contamination at each stage and the typical bioburden levels of the pre-irradiated tissue allografts. The standards expected of such laboratories are specified in Refs [2, 3].

The overall purpose of the above facilities contained within tissue banks is to demonstrate that the banks are capable of producing preserved tissue allografts that have acceptably low levels of microorganisms in the preserved product prior to their sterilization by radiation.

3.3. QUALIFICATION OF TISSUE DONORS

The main aim of the tissue donor selection process carried out prior to processing, preservation, storage and sterilization is to produce tissue allografts that are free from transmissible infectious diseases. Such a selection process to produce acceptable tissues shall include the following minimum items:

(a) The time of retrieval of the tissue after the death of the donor and the conditions of body storage;
(b) The age of the donor;
(c) The medical, social and sexual history of the donor;
(d) A physical examination of the body;
(e) Serological (including molecular biology) tests;
(f) Analysis of the autopsy as required by law.

Such information shall be used to screen donors in order to minimize the risk of infectious disease transmission from tissue donors to the recipients of the allografts. The information so collected shall be comprehensive, verifiable and auditable following good practice on tissue banking.

The following serological tests shall be carried out as a minimum on each donor:

(i) Antibodies to human immunodeficiency virus 1 and 2 (HIV 1, 2).

(ii) Antibodies to hepatitis C virus (HCV).
(iii) Hepatitis B surface antigen (HBs-Ag).
(iv) Syphilis: non-specific (e.g. VDRL) or, preferably, specific (e.g. TPHA).

Other tests may be required by statutory regulations or when specific infections are indicated. In using such laboratory based tests to provide additional assurance that allografts are free of transmissible disease, due consideration should be given to the detection limits of such tests. It should therefore be verified that the combination of processing, preservation and irradiation is capable of reducing the low levels of viral contamination that might be implied by an otherwise negative test to a SAL of 10^{-6}.

When addressing the problem of viral contamination, the same basic principles already advanced for the elimination of bacterial contamination need to be applied with regard to donor screening, serology, processing, preservation and sterilization by ionizing radiation. It should be noted that, in general, the D_{10} values for viruses are higher than those for bacteria and other microflora.

3.4. QUALIFICATION OF TISSUE PROCESSING AND PRESERVATION

The processing of tissue allograft materials such as bone, cartilage, ligaments, fascias, tendons, dura mater, heart valves and vessels, skin and amnion comprises various stages, such as removal of bone marrow, defatting, pasteurization, antibiotic treatment, percolation and treatment with disinfectants such as hypochlorite, ethyl alcohol and glycerol.

The inclusion of any or all of these stages will depend on a number of factors, including:

(a) The preferred practice of the tissue bank;
(b) The nature of the tissue (and its anticipated use in the clinic);
(c) The degree of contamination of the procured tissue.

The preservation of the processed tissue allografts may include:

(i) Freeze drying;
(ii) Deep freezing;
(iii) Air drying;
(iv) Heat drying;
(v) Chemical treatment.

An important function of the processes described in Sections 3.2–3.4 is to produce tissue allografts that have low levels of microbial contamination and, in particular, less than 1000 cfu (colony forming units) per allograft product when it is desired to substantiate a sterilization dose of 25 kGy. In the latter case, for a bioburden of 1000 cfu per allograft product, a 25 kGy dose is sufficient to achieve a SAL of 10^{-6} for an SDR (a reference microbial resistance distribution [1]). It should be recognized that microflora can originate from both the environment and the donor, and, in the case of the latter, may show substantial variation from donor to donor. The capacity of all of the tissue processing and preservation procedures to remove microorganisms should be checked periodically and documented.

3.5. MAINTENANCE OF VALIDATION

For each of the qualifications detailed in Sections 3.2–3.4, a validation process that will demonstrate that the standards expected will be maintained should be specified. As a minimum, these validation processes shall include:

(a) An audit of the origin and history of the procured tissues with reference to Section 3.3.
(b) A random, statistically significant sampling of procured tissues (i.e. prior to processing and preservation) followed by a laboratory based screening for viruses and infectious agents (see section 6.3 of Ref. [1]).
(c) Measures of particle count and microbial contamination in the environment of each of the separate facilities of the tissue bank.
(d) Random, statistically significant sampling of tissue allografts prior to and after tissue processing and preservation for measurements of bioburden levels.
(e) Determination of the ability of the tissue processing and preservation procedures to both reduce the levels of microorganisms and produce the levels of bioburden required for the radiation sterilization process. This should ensure a microbial contamination level of 1000 cfu per allograft product or less when substantiation of a sterilization dose of 25 kGy is required.

3.6. PROCESS SPECIFICATION

A process specification shall be established for each tissue allograft type. The specification shall include:

(a) The tissue allograft type covered by the specification;
(b) The parameters covering the selection of tissue for processing;
(c) Details of the tissue processing and preservation carried out prior to irradiation as appropriate for each tissue type;
(d) Details of the equipment, laboratory and storage facilities required for each of the processing and preservation stages, particularly with regard to acceptable contamination levels;
(e) Details of the routine preventative maintenance programme;
(f) Process documentation identifying every processed tissue, including details of its origin (see Section 3.3), its processing and preservation, dates of performance of all processes, details of process interruptions, and details of any deviations from the adopted processing and preservation procedures.

4. VALIDATION OF THE STERILIZATION PROCESS

4.1. GENERAL

The guidance given in this section is based on the procedures specified in Refs [1, 4–6] for the sterilization of health care products. More emphasis is given here, however, on the factors that affect the ability of the sterilization process to demonstrate that an appropriate SAL can be achieved with low numbers of tissue allografts. There may be more variability in the types and levels of microbial contamination than is found in health care products, which may also be variable in size and shape. More specifically, several approaches to establishing a sterilization dose are proposed for the small numbers of tissue allografts typically processed.

Emphasis is placed on the need to take into account both the variability of bioburden from one tissue donor to another and the variability of size and shape of tissue allografts, which can affect both the accuracy of product dose mapping (and hence the sterilization dose itself) and the applicability of using sample item portions (SIPs) of a tissue allograft product.

Validation of the sterilization process shall include the following elements:

(a) Qualification of the tissue allografts and their packaging for sterilization;
(b) Qualification of the irradiation facility;
(c) Process qualification using a specified tissue allograft or simulated products in qualified equipment;
(d) A certification procedure to review and approve documentation of (a–c);
(e) Activities performed to support maintenance of validation.

4.2. QUALIFICATION OF TISSUE ALLOGRAFTS FOR STERILIZATION

4.2.1. Evaluation of the tissue allograft and packaging

Prior to using radiation sterilization for a tissue allograft, the effect that radiation will have on the tissue allograft and its components shall be considered. The publications listed in the Bibliography contain information on this aspect. Similarly, the effect of radiation on the packaging shall also be considered. Guidance on the latter is given in annex I of Ref. [1]. Using such information, a maximum acceptable dose shall be established for each tissue allograft and its packaging.

4.2.2. Sterilization dose selection

A knowledge of the number and resistance to radiation of the microorganism population as it occurs on the tissue allografts shall be obtained and used for determination of the sterilization dose. For the sterilization of health care products, a reference microbial resistance distribution was adopted for the microorganisms found typically on medical devices [1].

Studies should be carried out to establish the types of microorganism that are normally found on the tissue types to be sterilized, as well as their numbers and resistance to radiation. Such studies should take account of the distribution of the microorganisms within the tissue allograft itself, since this may not be uniform. This should be determined by taking SIPs of the tissue and demonstrating that there are no significant statistical variations in distribution from SIP to SIP.

If such studies show a consistent distribution of microorganisms from one tissue allograft to another, and one which is less resistant than the SDR (see Table III–1), then a table similar to table B24 in Ref. [1] giving a distribution of

resistances appropriate to the allografts may be constructed for the purpose of sterilization dose setting. This would allow the use of appropriate and perhaps lower sterilization doses than would be the case if method 1, based on the SDR in Table III–1, were used [1]. In the absence of such studies, the SDR may be used to establish sterilization doses.

To establish a sterilization dose that will give a SAL of 10^{-6}, the methods based on those in Refs [1, 4–6] should be used. A summary of these approaches as they apply to tissue allografts is given in Annex I.

4.2.3. Technical requirements

The technical requirements to generate the information required for selection of the sterilization dose shall be:

(a) Access to qualified microbiological and dosimetric laboratory services;
(b) Microbiological testing performed in accordance with Refs [2, 3];
(c) Access to a ^{60}Co or ^{137}Cs radiation source or electron beam or X ray irradiators.

4.2.4. Transfer of sterilization dose

The conditions for transferring the procedures for the sterilization dose between two irradiation facilities are the same as those given in Ref. [1] (section 6.2.3) and apply equally to tissue allografts.

4.3. QUALIFICATION OF THE IRRADIATION FACILITY

The principles covering the documentation of the irradiation system, its testing, calibration and dose mapping are covered in Ref. [1] (section 6.3) and apply equally to tissue allografts.

4.4. QUALIFICATION OF THE IRRADIATION PROCESS

4.4.1. Determination of the product loading pattern

The principles given in Ref. [1] (section 6.4.1) covering the product loading pattern shall also apply for the sterilization of tissue allografts.

4.4.2. Product dose mapping

In general, the guidelines given in Ref. [1] (section 6.4.2) apply also to tissue allografts. However, it should be recognized that the product dose mapping of relatively uniform (i.e. in shape, size, composition and density) health care products is a more straightforward task than the product dose mapping of tissue allografts, which by their nature are more variable in their physical characteristics. In particular, the density of tissue allografts may vary depending on their water content.

In addition, some tissue allografts may be heterogeneous in their distribution of density within the product, requiring an appropriate number of dosimeters for the dose mapping exercise. A consideration of these factors affecting the actual absorbed dose in tissue allografts must be undertaken so that the level of accuracy in delivering a dose to a particular tissue can be determined.

The acceptability of the accuracy of delivering a dose to tissue allografts will depend on the dose delivered in the verification dose experiments. If, for example, the actual dose delivered at its lowest possible accuracy limit is less than 90% of the verification dose, then the verification test must be repeated at a higher dose.

Similarly, the minimum absorbed dose administered for sterilization should take into account the likely variation in dose delivered so that sterilization can be assured. As a guideline, uncertainties in the delivered dose should be within ±10%.

4.5. MAINTENANCE OF VALIDATION

The guidelines covering calibration of equipment and dosimetric systems, irradiator requalification and sterilization dose auditing are the same as given in Ref. [1] (section 6.6) and apply equally to tissue allografts.

4.6. ROUTINE STERILIZATION PROCESS CONTROL

The guidelines covering process specification, tissue allograft handling and packing in the irradiation container, and sterilization process documentation are similar to those given in Ref. [1] (section 7) and apply equally to tissue allografts.

4.7. QUALITY, SAFETY AND CLINICAL APPLICATION OF THE TISSUE ALLOGRAFT

A programme to demonstrate the quality, safety and clinical application of the tissue allograft throughout its shelf life shall be performed. Sampling procedures appropriate to the tissue type should be devised for this purpose.

4.8. DOCUMENTATION AND CERTIFICATION PROCEDURES

Information gathered or produced while conducting the qualification and validation of the tissue allografts, tissue bank facilities and tissue processing, preservation and radiation sterilization procedures shall be documented and reviewed for acceptability by a designated individual or group and retained in accordance with Ref. [9].

4.9. MANAGEMENT AND CONTROL

Control of the procedures involved in the selection of tissue donors, tissue processing and preservation prior to sterilization by radiation, and the radiation sterilization process itself, shall be fully documented and managed in accordance with Ref. [9].

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Annex I

ESTABLISHING A STERILIZATION DOSE

I–1. SCOPE

This annex describes the practices and procedures for determining the bioburden levels of tissue allografts and the application of this information to establish the radiation sterilization dose.

I–2. SELECTION OF TISSUE ALLOGRAFT PRODUCTS

Tissue allografts can be prepared from a wide range of tissues, such as skin, amnion, bone, cartilage, tendons and ligaments. If samples can be prepared from these tissues that are reasonably reproducible in shape, size and composition and also in sufficient numbers for statistical purposes, then the usual sampling procedures apply, as given, for example, in Refs [I–1, I–2]. However, if allograft products are few in number (less than ten) and cannot be considered as identical products, then it may be necessary to take multiple SIPs of a single tissue allograft product for both bioburden analysis prior to sterilization and for the purpose of establishing a sterilization dose. In such instances, it is important to have confidence in the distribution of microorganisms throughout the sample, obtained, for example, by periodic monitoring of such products.

I–3. SAMPLE ITEM PORTION

The SIP shall validly represent the microbial challenge presented to the sterilization process. SIPs may be used to verify that microorganisms are distributed evenly, for bioburden estimation and for establishing a sterilization dose. It is important to ascertain that the SIPs are representative, not only in shape, size and composition but also in bioburden. Statistical tests should be applied to establish this. At least 20 SIPs should be used (ten for bioburden testing and ten for the verification dose experiments).

I–4. BIOBURDEN DETERMINATION

Bioburden determination could include a count of aerobic bacteria, spores, yeasts, moulds and anaerobic bacteria. Many factors determine the choice of the tests most appropriate for the tissue allograft. At a minimum, the aerobic bacteria and fungi should be counted.

The objective of the bioburden determination is to:

(a) Determine the total number of viable microorganisms within or on a tissue allograft and the packaging after completion of all processing steps before sterilization;
(b) Act as an early warning system for possible production problems;
(c) Calculate the dose necessary for effective radiation sterilization.

The validation of the bioburden estimation requires determination of the effectiveness and reproducibility of the test method.

The steps to estimate bioburden are shown in Fig. I–1; full details can be found in Ref. [I–3].

I–5. DETERMINATION OF THE VERIFICATION DOSE

I–5.1. Verification dose experiments

In Ref. [I–1] the concept of establishing a verification dose for a SAL value that is much higher than 10^{-6}, for example for a SAL value of 10^{-2}, was proposed as an experimental method of establishing the sterilization dose corresponding to a SAL of 10^{-6}.

For such verification dose experiments, samples of tissue allografts should be taken from production batches and irradiated at the calculated verification dose. In these experiments it is assumed (and should be demonstrated statistically) that the tissue allograft products are reasonably uniform in shape, size, composition and bioburden distribution. For single batch sizes up to 999, the number of samples required may be obtained from table 1 in Ref. [I–2]. For minimum batch sizes of 20–79, for example, ten samples are required for the bioburden determination and ten for the verification dose experiment. In general, the number of samples required for the bioburden determination and verification dose experiments will depend on the number of batches and the number of samples in each batch. For each circumstance, the number of positive sterility tests allowed in the verification dose experiment should be calculated statistically using an acceptable range of values of probability

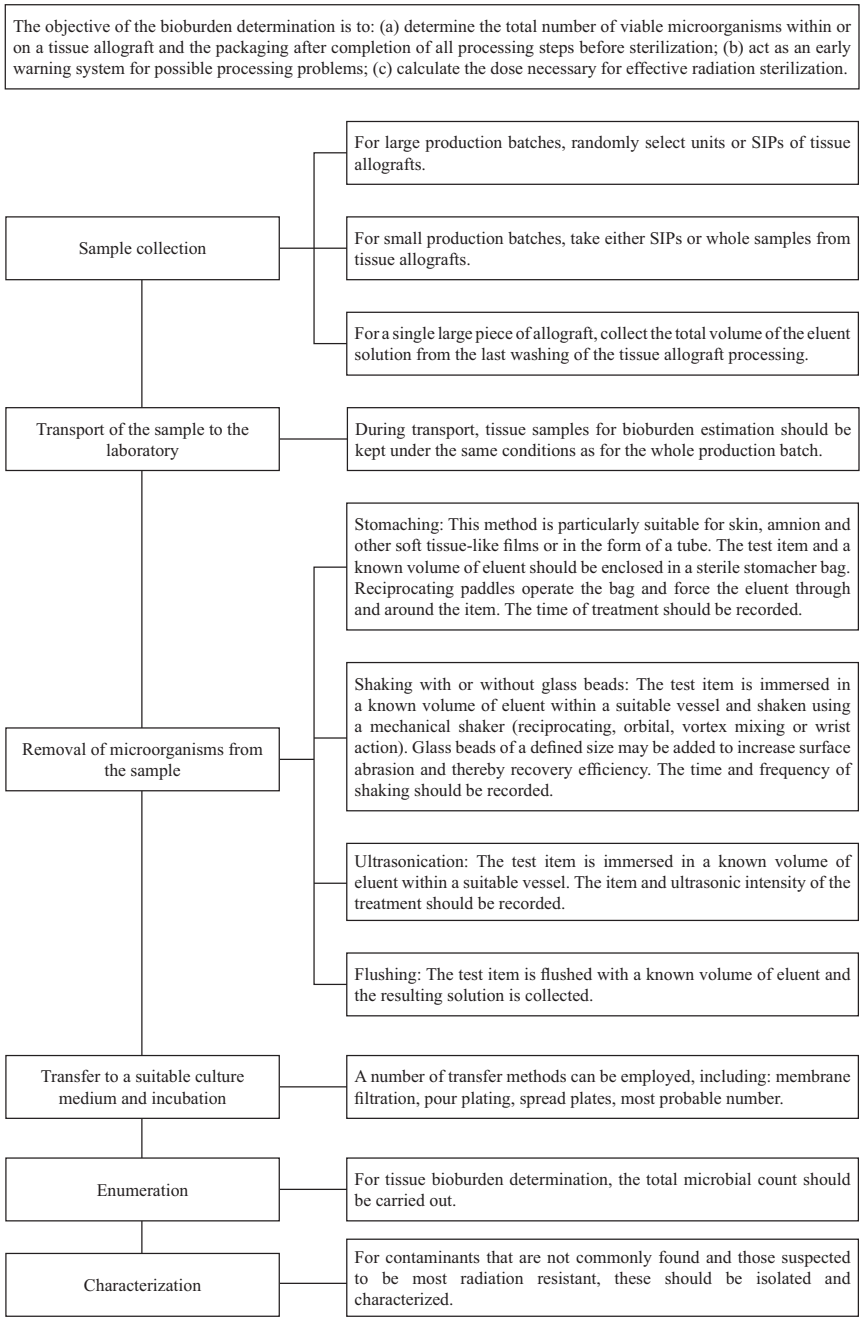

FIG. I–1. Steps in the estimation of the bioburden.

for 0, 1, 2, 3, etc., positive tests of sterility. For the 100 samples used in method 1 in Ref. [I–1], for example, there is a 92% chance of there being 1% positives when up to two positives are detected and a 10% chance of accepting a batch with 5.23% positives [I–4].

For the ten samples taken in Ref. [I–2] from a batch of 20, up to one positive test of sterility is proposed. For 30 or more, up to two positive tests of sterility are proposed [I–2]. It should be noted that these latter statistical tests do not offer the same degree of protection as obtained when accepting up to two positive tests of sterility for a sample size of 100; for example, when accepting up to one positive test of sterility in a sample size of ten, there is a 95% chance of accepting a batch with 3.68% positives and a 10% chance of accepting a batch with 33.6% positives. Alternative sampling strategies are now available [I–4] that include, for example, double sampling plans that can minimize sample sizes and yet offer similar protection. For single batches of low sample sizes, protection levels similar to those of the 100 sample approach in Ref. [I–1] can only be obtained by accepting a small number (possibly even zero) of positive sterility tests (e.g. accepting up to one positive for a sample size of 50 offers similar protection).

Hence, in Ref. [I–2] the verification dose for ten samples taken from a batch of 20 is that required to produce a SAL of 10^{-1} (the reciprocal of the number of SIPs used) and is that dose which will yield not more than one positive test of sterility from the ten irradiated SIPs.

I–5.2. Selection of dose setting method

In order to calculate the verification doses as well as the doses required to produce a SAL value of 10^{-6}, one of several approaches may be taken to establish an appropriate verification dose for low sample numbers (up to 100, but typically much less). The methods proposed here for the establishment of a sterilization dose are based on statistical approaches used previously for the sterilization of health care products [I–1, I–2, I–5, I–6] and modified appropriately for the typically low numbers of tissue allografts samples available. For an SDR, the tissue bank may elect to substantiate a sterilization dose of 25 kGy for microbial levels up to 1000 cfu per unit. Alternatively, for the SDR and other microbial distribution, specific sterilization doses may be validated depending on the bioburden levels and radiation resistances (D_{10} values) of the constituent microorganisms. The following shows the available methods:

— Method A. For establishing specific sterilization doses for an SDR and other microbial distributions for samples sizes between 10 and 100, an adaptation of method 1 in Ref. [I–1] may be used. Method 1 in Ref. [I–1]

is normally used for multiple batches containing a large number of samples per batch. For batches of 100 samples, for example, verification dose experiments are carried out for a SAL of 10^{-2}. A successful experiment (up to two positive tests of sterility) will then enable the dose required to achieve a SAL value of 10^{-6} to be calculated from the survival curve of an SDR. In this code of practice, an extension of table 1 in Ref. [I–1] is given so that verification doses for SAL values between 10^{-2} and 10^{-1} may be found for bioburden levels up to 1000 cfu per allograft product. These SAL values correspond to relative low sample sizes of 10–100. This allows method 1 to be used for typical tissue allografts for which relatively low numbers of samples are available and the distribution of microbial radiation resistances is known and is different to the SDR. The worked example given later uses this approach and, in addition, applies it (with appropriate statistical sampling, see above) to a microbial population that has a different distribution of radiation resistances than the SDR. However, for low bioburden levels combined with low sample numbers, it may be anticipated that there is an increased probability when using this adaptation of method 1 that the verification dose experiment may fail. In the case of failure, the methods outlined in methods B and/or C may be used.

— Method A(i). A similar approach can also be undertaken when the distribution of microbial radiation resistances is known and is different to the SDR. The worked example given in Annex II uses this approach and, in addition, applies it (with appropriate statistical sampling, see above) to a microbial population that has a different distribution of radiation resistances than the SDR. However, it should be noted that, for both methods A and A(i), low bioburden levels combined with low sample numbers will give rise to an increased probability of failure of the verification dose experiment. In the event of failure, methods B and C for substantiation of a 25 kGy sterilization dose may decrease this risk.
— Method B. For substantiation of a 25 kGy sterilization dose, the method in Ref. [I–2] may be used to calculate the verification dose. This is an accredited method and is essentially a modification of method A and applies only to an SDR. In this method, the verification dose for a given SAL is approximated to the initial bioburden by a series of linear relationships. Each linear equation is valid for a particular tenfold domain of bioburden level, for example 1–10 cfu. The method in Ref. [I–2] can only be used to substantiate a dose of 25 kGy. It should be noted that the statistical approach allowing up to one positive test for sample sizes up to 30 and up to two positive tests for sample sizes above 30 does not offer the same level of protection as for the 100 samples in ISO 11137 [I–1]

until the sample size reaches 100. Alternative sampling strategies may be employed [I–4] for all the verification dose methods proposed here.

— Method C. For substantiation of a 25 kGy sterilization dose, an alternative and more recent method given in Ref. [I–6] may be used. The modification takes into account how the verification dose varies with bioburden level for a given SAL (and sample size) on the assumption that a SAL of 10^{-6} is to be achieved at 25 kGy. Depending on the actual bioburden levels to be used (1–50 or 51–1000 cfu per allograft product), a linear extrapolation of the appropriate SDR survival curve is made from either (log N_0, 0 kGy) or (log 10^{-2}) to (log 10^{-6}, 25 kGy) for 1–50 cfu and 51–1000 cfu, respectively. For bioburden levels of less than 1000 cfu per allograft unit, these constructed survival curves represent a more radiation resistant bioburden than would otherwise be the case. The validity of this approach arises from the purpose of the method, which is to validate a sterilization dose of 25 kGy. For all bioburden levels below 1000 cfu per allograft product, this means that for the reference microbial resistance distribution given in table B24 in Ref. [I–1] for medical devices, a more conservative approach to the calculation of a verification dose is taken. Hence, this modification allows the use of greater verification doses than would be allowed using the formula given in either method 1 in Ref. [I–1] or Ref. [I–2]. The result is that there are fewer unexpected and unwarranted failures relative to verification dose experiments carried out using the method in Ref. [I–2]. At a bioburden level of exactly 1000 cfu per allograft product (the maximum in both methods), there is no difference in the outcomes of the methods (i.e. the calculated verification doses are identical).

I–6. PROCEDURES

I–6.1. Establish test sample sizes

Select at least ten allograft products or SIPs, as appropriate, for determination of the initial bioburden. The number of allograft products or SIPs should be sufficient to represent validly the bioburden on the allograft products to be sterilized.

Select between ten and 100 allograft products (or SIPs) for the verification dose experiments and record the corresponding verification dose SAL (= $1/n$, where n is the number of allograft products or SIPs used).

I–6.2. Determine the average bioburden

Using methods such as those in Ref. [I–3] and as described above (bioburden estimation), determine the average bioburden of at least ten allograft products or SIPs (the number will depend on the number of batches and the number of samples in the batches). For SIP values less than unity, the bioburden level for the whole product should be calculated and should be less than 1000 cfu per allograft product for verification dose experiments carried out to substantiate a 25 kGy sterilization dose.

I–6.3. Establish the verification dose

The appropriate verification dose depends on the number of samples (allograft products or SIPs) to be used in the experiment (= 1/number of samples). The verification dose calculation depends on which of the three methods above is being used, as follows:

— Methods A and A(i). For establishing specific sterilization doses for SDR and other microbial distributions for samples sizes between ten and 100 (an adaptation of method 1 in Ref. [I–1]). Calculate the dose required to achieve the required SAL from knowledge of the initial bioburden level and from the microbial distribution and associated radiation resistances. This may be calculated from the equation:

$$N_{\text{tot}} = N_{0(1)}\, 10^{-(D/D1)} + N_{0(2)}\, 10^{-(D/D2)} + \ldots + N_{0(n)}\, 10^{-(D/D(n))}$$

where N_{tot} is the number of survivors, $N_{0(i)}$ is the initial number of the various microbial strains i (where $i = 1 - n$), $D1$, $D2$, $D(n)$ are the D_{10} values of the various microbial strains, D is the radiation dose and n is the number of terms in the equation for an SDR ($n = 10$).

- Method A. For the reference SDR used in Ref. [I–1] for medical devices (see Table III–1), this equation will produce data similar to table B1 in Ref. [I–1] but for SAL values between 10^{-2} and 10^{-1} instead. By equating N_{tot} to the selected SAL value and by using the appropriate D_{10} values for each microbial type together with their numbers prior to irradiation, the verification dose D for SAL values between 10^{-2} and 10^{-1} can be calculated. These values are set out in the tables in Annex III. The same calculation can be used to find the sterilization dose for the desired SAL of 10^{-6}, or reference can be made to table B1 in Ref. [I–1]. In this method, the sterilization dose is calculated using the bioburden level of the whole product.

Alternatively, approximate values of the verification doses to achieve the same SAL values may be calculated using the equation given in Ref. [I–2].

- Method A(i). The same equation above can be adopted to calculate both the verification and sterilization doses where a distribution of microflora that is different to the SDR is to be sterilized. It requires a knowledge of the different proportions of microflora with their respective D_{10} values. A worked example is given in Annex II.

— Method B. For substantiation of a 25 kGy sterilization dose: From a knowledge of the average bioburden and the number of samples or SIPs to be used in the verification experiment, the verification dose for an SDR is approximated by the equation:

Verification dose at the selected SAL = $I + [S \times \log (\text{bioburden})]$

where I and S are given in Table III–9 in Annex III of this code of practice.

— Method C. For substantiation of a 25 kGy sterilization dose [I–6]: The calculation of the verification dose follows the procedures in Ref. [I–7], where the bioburden levels refer to either the SIP or the whole product, whichever is being used in the verification dose experiment.
For bioburden levels of 1–50 cfu per allograft product or SIP:

Step 1: $D_{\text{lin}} = 25 \text{ kGy}/(6 + \log N_0)$

Step 2: Verification dose = $D_{\text{lin}} (\log N_0 - \log \text{SAL}_{\text{VD}})$

where D_{lin} is the D_{10} dose for a hypothetical survival curve that is linear between the coordinates ($\log N_0$, 0 kGy) and ($\log 10^{-6}$, 25 kGy) for initial bioburden levels N_0 up to 1000 cfu per allograft product. This linear plot therefore represents a constructed survival curve in which there is a 1 out of 10^6 probability of a survivor at 25 kGy. The method is therefore valid only for the substantiation of a 25 kGy sterilization dose, regardless of whether a lower dose could in fact be validated.
For bioburden levels of 51–1000 cfu per allograft product or SIP:

Step 1: For a particular value of bioburden, use table B1 in Ref. [I–1] to identify the doses (kGy) corresponding to SAL values of 10^{-2} [$D(10^{-2})$]

and 10^{-6} [$D(10^{-6})$]. From these values, calculate TD_{10} from the following equation:

$$TD_{10} = (Dose_{-6}\ kGy - Dose_{-2}\ kGy)/4$$

where TD_{10} represents the hypothetical D_{10} value for a survival curve for an SDR that has been modified such that it is linear between log 10^{-2} and log 10^{-6} (log SAL values) when plotted against dose, with the log 10^{-6} value being set at 25 kGy. Essentially, this produces a survival curve that is more resistant to radiation than the SDR (for bioburden levels of less than 1000 cfu per allograft product) and one that is appropriate to substantiation of a 25 kGy sterilization dose only.

Step 2: Verification dose = 25 kGy – [TD_{10} (log SAL_{VD} + 6)]

where SAL_{VD} is the SAL at which the verification dose experiment is to be performed.
In Ref. [I–6] a refinement of the above calculations has been undertaken, and as a result values of verification doses for a SAL of 10^{-1} for bioburden values between 0 and 1000 cfu can be found in tabular form in that publication — they are reproduced in Annex III. For other SAL values the methods of calculation detailed above should be used. In Ref. [I–6] the verification dose for SIP values less than unity are calculated from the equation:

SIP verification dose + (SIP = 1 verification dose) + (log SIP × SIP dose reduction factor)

Values of the SIP dose reduction factor can be found in Table III–10 in Annex III (for verification dose experiments conducted at a SAL of 10^{-1}).

I–6.4. Perform verification dose experiment

Irradiate the tissue allografts or SIPs thereof at the verification dose. The irradiation conditions of the samples for verification of the substerilization dose should be the same as for the whole batch that is to be sterilized; for example, if the produced tissue batch is irradiated in a frozen condition, the samples for the substerilization dose verification studies should be irradiated in the same condition and the frozen condition should be kept during the whole irradiation process.

The defined test sample size (SIP (1), according to the SAL and batch size, is exposed to radiation at the verification dose. The dose delivered should not be less than 90% of the calculated verification dose.

Test the tissue allografts for sterility using the methods in Ref. [I–8] and record the number of positive tests of sterility. The irradiated SIPs, of all types of tissue allografts, are transferred to a growth medium and incubated for at least 14 days at appropriate temperatures. Positive and negative sterility test results should be registered. For bone and skin allografts, an additional test is recommended to detect anaerobic bacteria.

I–6.5. Interpretation of results

For a verification dose experiment performed with up to 30 allograft products or SIPs, statistical verification is accepted if no more than one positive test of sterility is observed. For 30–100 products or SIPs, statistical verification is accepted if no more than two positive tests of sterility are observed [I–2]. However, it should be noted that the degree of protection in accepting these limits varies according to the sample size taken (see above).

Where the verification dose experiment is successful, the dose required to produce a SAL of 10^{-6} for the whole allograft product should be calculated for method A, as indicated above and calculated in Table III–3 in Annex III.

For methods B and C, a successful verification dose experiment substantiates the use of 25 kGy as a sterilization dose.

I–7. ROUTINE USE OF STERILIZATION DOSES

The routine use of a sterilization dose calculated with method A or of 25 kGy as substantiated by either method B or C shall only be valid if the tissue selection and tissue processing procedures have been demonstrated to produce tissue allografts with consistent bioburden levels. It should be demonstrated that the level of variation in bioburden is consistent with the sterilization dose to be used routinely. In such cases, sterilization dose audits should be carried out at regular intervals of at least every three months.

REFERENCES TO ANNEX I

[I–1] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, Sterilization of Health Care Products — Requirements for Validation and Routine Control — Radiation Sterilization, ISO 11137: 1995, ISO, Geneva (1998).

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Annex II

STERILIZATION OF TISSUE ALLOGRAFTS

II–1. EXAMPLES OF STERILIZATION PROCEDURES

This is a case of a limited number of amnion samples with a low bioburden and a low bacterial resistance, using method 1 in Ref. [II–1] to calculate the verification dose.

II–1.1. Introduction

This method uses method A(i), an adaptation of method 1 found in Ref. [II–1], but applies it to sample sizes of less than 100 in a single production batch. The example chosen consists of a single batch of 20 amnion membranes (5 × 5 cm) from which ten are used for the bioburden determination and ten are used for the verification dose experiment. The data used in the example are consistent with data on bioburden levels, bacterial types and distribution found in Ref. [II–2]. In that study, the most radiation resistant microbes were assumed to have a D_{10} value of 1.8 kGy (i.e. a distribution that differs from the reference microbial resistance distribution in that there are no microbes with a D_{10} value higher than 1.8 kGy). Furthermore, the tissue processing and preservation procedures have produced tissue allografts that have much less than 1000 cfu per allograft product. For such samples, a sterilization dose that is significantly less than 25 kGy is confirmed from the verification dose experiment.

II–1.2. Procured tissue qualification

(a) Tissue type: amnion samples of 5 × 5 cm.
(b) Screening of tissue for transmission of disease. Age of donor: 25. Medical, social and sexual history: none to suggest risk of transmissible disease. Serological tests: HIV (HIV 1, 2 Ab): negative; hepatitis C (HCV-Ab): negative; hepatitis B (HBs-Ag): negative; syphilis (VDRL): negative.

II–1.3. Tissue processing and preservation qualification

(a) Description of processing technique: hypochlorite.
(b) Description of preservation technique: lyophilization.
(c) Typical microbial levels of procured tissue before processing: in the range of 5000–10 000 cfu per tissue.

(d) Typical bioburden levels of processed and preserved tissues: 57 cfu per allograft product.[1]

II–1.4. Qualification of tissue allografts for sterilization

A typical bioburden distribution is assumed. The distribution of bacterial resistances given below is assumed to consist entirely of bacteria with a D_{10} value of 1.8 kGy and represents a distribution that is similar but not identical to the SDR; that is:

D_{10} (kGy): 1.8

Frequency: 1.0

The calculation of the sterilization dose is given in Table II–1.

II–1.5. Conclusion

This example shows how a combination of good tissue processing and preservation and sterilization by ionizing radiation, for samples that are known to have bacterial contamination relatively susceptible to radiation, can allow the use of a sterilization dose that is much less than 25 kGy.

[1] It is noted from the study of Hilmy et al. [II–3] that the bioburden levels of the processed tissue (i.e. before sterilization by irradiation) decreased from about 1400 cfu to 120 cfu during the study period 1994–1997, with 1998 data showing an average of 57 cfu per allograft product (range 12–160 cfu). Clearly, good processing techniques can have a dramatic effect on the bioburden levels of the tissue being prepared for sterilization by irradiation. The level of reduction used in this example is probably therefore a conservative estimate of the degree of elimination of bacteria.

TABLE II–1. CALCULATION OF THE STERILIZATION DOSE

Stage	Value	Comments
Stage 1		
Production batch size	40	5 × 5 cm amnion samples
Test sample size for bioburden determination	10	
Test sample size for the verification dose experiment	10	Verification dose required for SAL 10^{-1} (= 1/10)
Stage 2		
Obtain samples	20	Ten for bioburden, ten for verification dose experiment
Stage 3		
SIP	1	The whole allograft product is used
Average bioburden	57	Bioburden results of 15, 91, 99, 30, 30, 99, 8, 84, 91 and 23
Stage 4		
Verification dose calculation	4.96 kGy	Using the bacterial resistance distribution given above (and not the SDR), the survival equation is constructed (see Annex I) and used to calculate the verification dose (D) for an N_{tot} value of 0.1 (equivalent to a SAL value of 0.1, the reciprocal of the number of samples used) and where the total initial number of microorganisms per product (SIP = 1) is equal to 57 The survival equation is: $N_{tot} = 57 \times 10^{-(D/1.8)}$ From these data, the verification dose is calculated as 4.96 kGy

TABLE II–1. CALCULATION OF THE STERILIZATION DOSE (cont.)

Stage	Value	Comments
Stage 5		
Verification dose experiments	5.0 kGy (delivered dose) One positive/ ten samples	The sterility test yielded one positive test out of ten, and therefore the verification dose experiment was successful (but note that the level of protection is significantly less than allowing up to two positives for a sample size of 100, see Annex I) and the sterilization dose for SAL = 10^{-6} can be calculated from the survival equation given above (= 14.0 kGy) Note: In the case that a SIP < 1 was taken instead, the bioburden for the whole product should be used to calculate the sterilization dose

II–2. LIMITED NUMBER OF AMNION SAMPLES REQUIRING ONLY SUBSTANTIATION OF 25 kGy AS A STERILIZATION DOSE

II–2.1. Introduction

In this example it is assumed that there is an SDR that defines the bacterial contamination of the tissue allografts. The example chosen consists of a single batch of 40 amnion membranes (5 × 5 cm) from which ten are used for the bioburden determination and ten are used for the verification dose experiment. The data used in the example are consistent with data on bioburden levels, bacterial types and distribution found in Ref. [II–3]. Furthermore, for the limited number of samples to be tested, it is required only to establish that a 25 kGy dose may be used to achieve a SAL of 10^{-6}. It is shown below that when the method in Ref. [II–2] is applied for 20 samples (ten for the bioburden determination and ten for the verification dose experiment), from a batch size of 40, the samples fail the verification dose experiment. To increase the probability of a successful verification dose experiment, while at the same time substantiating a sterilization dose of 25 kGy, method C (based on the method of Tallentire [II–4]) is applied (see Annex I). This allows the use of a higher verification dose and it is then found that the samples pass this test, substantiating the use of a 25 kGy sterilization dose.

II–2.2. Procured tissue qualification

(a) Tissue type: amnion (5 × 5 cm).
(b) Screening of tissue for transmission of disease. Age of donor: 25. Medical, social and sexual history: none to suggest risk of transmissible disease. Serological tests: HIV (HIV 1, 2 Ab): negative; hepatitis C (HCV-Ab): negative; hepatitis B (HBs-Ag): negative; syphilis (VDRL): negative.

II–2.3. Tissue processing and preservation qualification

(a) Description of processing technique: hypochlorite.
(b) Description of preservation technique: lyophilization.
(c) Typical microbial levels of procured tissue before processing: in the range of 5000–10 000 cfu per tissue.
(d) Typical bioburden levels of processed and preserved tissue: 57 cfu per allograft product.[2]

II–2.4. Qualification of tissue allografts for sterilization

A typical bioburden distribution is assumed. Also it is assumed that the SDR (see Annex I) applies. The calculation of the sterilization dose is given in Table II–2.

II–2.5. Conclusion

Although the tissue processing and preservation processes produced tissues with relatively low bioburden for which sterilization doses substantially less than 25 kGy could have been used (see example above), the tissue bank required only a method to substantiate the use of a standard sterilization dose of 25 kGy. The application of the methods in Refs [II–2, II–4], which are particularly suitable for bioburden levels much less than 1000 cfu per allograft product, allowed the use of relatively high verification doses and hence a higher probability of success. In the example chosen, the method in Ref. [II–2] failed and hence the method in Ref. [II–4] was used as well. For tissue banks that prefer to use a standard 25 kGy sterilization dose, this latter method will be more efficient in that fewer verification dose experiments will fail.

[2] See Footnote 1.

TABLE II–2. TYPICAL BIOBURDEN DISTRIBUTION

(it is assumed that the standard distribution of resistance, see Annex I, is valid)

Stage	Value	Comments
Stage 1		
Production batch size	40	5 × 5 cm amnion samples
Test sample size for bioburden determination	10	
Test sample size for the verification dose experiment	10	Verification dose required for SAL 10^{-1} (= 1/10)
Stage 2		
Obtain samples	20	Ten for bioburden, ten for the verification dose experiment
Stage 3		
SIP	1	The whole allograft product is used
Average bioburden	57	Bioburden results of 15, 91, 99, 30, 30, 99, 8, 84, 91 and 23 The average bioburden for the whole product is 57 cfu (this is less than 1000 cfu and therefore the method may be used) Note: If a SIP < 1 was taken, then the bioburden of the whole product should be calculated and should be less than 1000 cfu per allograft product for this method to be valid
Stage 4		
Verification dose calculation (1)	4.6 kGy	The verification dose is calculated using the method in Ref. [II–2]. In this method (applicable to an SDR only), the verification dose for a given SAL is approximated to the initial bioburden by a series of linear relationships using the parameters *I* and *S* (see below). Each linear equation is valid for a particular tenfold domain of bioburden level, for example 10–100 cfu. For a bioburden of 57 and sample size of 10, *I* and *S* values of 0.67 and 2.23, respectively, are obtained from Ref. [II–2] and are given here in

TABLE II–2. TYPICAL BIOBURDEN DISTRIBUTION

(it is assumed that the standard distribution of resistance, see Annex I, is valid) (cont.)

Stage	Value	Comments
		Table III–9 in Annex III. The verification dose is given by: Dose = I + [S × log (average SIP bioburden)] = 0.67 + 2.23 × log (57)) = 4.6 kGy
Stage 5		
Verification dose experiments (1)	4.5 kGy (delivered dose) Two positives/ ten samples	The sterility test yielded two positive tests out of ten, and therefore the verification dose experiment was not successful and a sterilization dose of 25 kGy could not be substantiated
Verification dose calculation (2)	8.7 kGy	A new verification dose was calculated using the method in Ref. [II–4] (see Annex I). This method takes into account how the verification dose for an SDR (reference microbial resistance distribution) varies with bioburden level for a given SAL (and sample size) on the assumption that a SAL of 10^{-6} is to be achieved at 25 kGy. Application of method 1 in Ref. [II–1] for bioburden levels of less than 1000 cfu would yield sterilization doses of less than 25 kGy. The method in Ref. [II–4] assumes instead that only substantiation of a 25 kGy sterilization dose is required, regardless of the bioburden level. Extrapolation of the reference distribution to produce a SAL of 10^{-6} at 25 kGy for bioburden levels of less than 1000 cfu allows the use of higher verification doses than would be predicted by method 1 in Ref. [I–1] and hence a greater probability of a successful verification dose experiment. For a bioburden level of 57 (i.e. between 51 and 1000), the doses corresponding to this bioburden for SAL values of 10^{-6} and 10^{-2} are found from table 1 in Ref. [I–1] and are designated $Dose_{-6}$ and $Dose_{-2}$, respectively, from which TD_{10} is calculated as follows:

TABLE II–2. TYPICAL BIOBURDEN DISTRIBUTION

(it is assumed that the standard distribution of resistance, see Annex I, is valid) (cont.)

Stage	Value	Comments
		$TD_{10} = (Dose_{-6}\ kGy - Dose_{-2}\ kGy)/4$ $= (20.4 - 7.3)/4$ $= 3.27$ kGy Note: Table 1 in Ref. [I–1] does not have a value corresponding to a bioburden of 57 and so the next highest value of 57.2 is used. TD_{10} represents the hypothetical D_{10} value for a survival curve for an SDR that has been modified such that it is linear between log 10^{-2} and log 10^{-6} (log SAL values) when plotted against dose, with the log 10^{-6} value being set at 25 kGy. Essentially, this produces a survival curve that is more resistant to radiation than the SDR (for bioburden levels of less than 1000 cfu per allograft product) and one that is appropriate to substantiation of a 25 kGy sterilization dose only. The verification dose VD is then calculated as follows : $VD = 25\ kGy - [TD_{10}\ (\log SAL_{VD} + 6)]$ $= 25 - [3.27\ (\log 0.1 + 6)]$ $= 8.7$ kGy (note that table 4 in Ref. [II–5] gives a refined value of 8.9 kGy) SAL_{VD} is the SAL at which the verification dose experiment is to be performed (= the reciprocal of the number of samples), in this case 0.1
Verification dose experiments (2)	8.5 kGy One positive/ ten samples	The ten samples are irradiated at this verification dose and tested for sterility The sterility tests yielded one positive test out of ten, and therefore the use of 25 kGy as a sterilization dose (SAL = 10^{-6}) could be substantiated (note, however, that this result does not offer the same level of protection when allowing up to two positives in a sample size of 100, see above)

II–3. LIMITED NUMBER OF BONE SAMPLES WITH VERY LOW BIOBURDEN AND STANDARD DISTRIBUTION OF RESISTANCE, USING THE METHOD IN REF. [II–2] TO CALCULATE THE VERIFICATION DOSE (SIP < 1)

II–3.1. Introduction

This method uses the method in Ref. [II–2] and applies it to a sample of 40 small pieces of bone. Typically, very low bioburden levels are found after processing. In this example, very low SIP values are used so that most of the allograft product can be retained for use.

II–3.2. Procured tissue qualification

(a) Tissue type: bone cut into 40 small pieces (chips).
(b) Screening of tissue donor. Age of donor: 36. Medical, social and sexual history: none to suggest risk of transmissible disease. Serological tests: HIV (HIV 1, 2 Ab): negative; hepatitis C (HCV-Ab): negative; hepatitis B (HBs-Ag): negative; syphilis (VDRL): negative.

II–3.3. Tissue processing and preservation qualification

(a) Description of processing technique: cut into standardized small pieces.
(b) Description of preservation technique: frozen.
(c) Typical bioburden levels of processed and preserved tissues: 40 cfu per allograft product.

II–3.4. Qualification of tissue allografts for sterilization

The calculation of the sterilization dose is given in Table II–3.

II–3.5. Conclusions

Although a lower sterilization dose could be justified if the adaptation of method 1 in Ref. [II–1] was applied, the tissue bank elected to use the method in Ref. [II–2] to substantiate a 25 kGy sterilization dose only.

TABLE II–3. QUALIFICATION OF TISSUE ALLOGRAFTS FOR STERILIZATION

Stage	Value	Comments
Stage 1		
Production batch size	5	Bone cut into 40 small pieces (1 cm^3 each), packed in a flask, produced from one donor in one processing batch
Test sample size for bioburden determination	10	According to table 1 in Ref. [II–2]
Test sample size for the verification dose experiment	10	According to table 1 in Ref. [II–2]
Stage 2		
Obtained samples	20	A random sample of 20 standardized product portions of 1 cm^3 each was obtained from the production batch
Stage 3		
SIP	0.025	Calculated from 1/40
SIP bioburden	1	Bioburden results of 1, 0, 2, 0, 1, 2, 1, 1, 1 and 1 were observed from the ten SIPs tested, for an average bioburden of 1
Average bioburden	40	The average bioburden for the product tested was calculated as follows: 1/0.025 = 40. This is less than 1000 cfu per allograft product and therefore this method is valid.
Stage 4		
Verification dose calculation	1.3	Verification dose formula: $I + (S \times \log$ (average SIP bioburden) kGy According to table 2 in Ref. [II–2], the I and S values are 1.25 and 1.65, respectively: $= 1.25 + (1.65 \times \log 1)$ = 1.25 kGy = 1.3 kGy
Stage 5		
Verification dose experiment	1.3 kGy (delivered dose) Zero positive/ ten samples	The test sterility yielded zero positive from the ten SIPs tested, therefore the verification experiment was successful and no further action was necessary

TABLE II–3. QUALIFICATION OF TISSUE ALLOGRAFTS FOR STERILIZATION (cont.)

Stage	Value	Comments
Stage 6		
Interpretation of results		The test of the sterility result was acceptable; the sterilization dose of 25 kGy was confirmed

REFERENCES TO ANNEX II

[II–1] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, Sterilization of Health Care Products — Requirements for Validation and Routine Control — Radiation Sterilization, ISO 11137: 1995, ISO, Geneva (1998).

[II–2] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, Sterilization of Health Care Products — Radiation Sterilization — Substantiation of 25kGy as a Sterilization Dose for Small or Infrequent Production Batches, Rep. ISO/TR 13409: 1996, ISO, Geneva (1996).

[II–3] HILMY, N., FEBRIDA, A., BASRIL, A., Validation of radiation sterilization dose for lyophilized amnion and bone grafts, Cell Tissue Bank **1** (2000) 143–148.

[II–4] KOWALSKI, J.B., TALLENTIRE, A., Substantiation of 25kGy as a sterilization dose: A rational approach to establishing verification dose, Radiat. Phys. Chem. **54** (1999) 55–64.

Annex III

STANDARD DISTRIBUTION OF RESISTANCE VALUES AND RADIATION DOSES NECESSARY TO ACHIEVE GIVEN VALUES OF STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDEN LEVELS

The tables in this annex are either taken from other publications, as referenced, or have been calculated specifically for the present recommendations. The latter tables contain a series of SDR values and radiation doses necessary to achieve given values of SAL for different bioburden levels, calculated using the equation described for method B in Section I–6.

REFERENCES TO ANNEX III

[III–1] DAVIS, K.W., STRAWDERMAN, W.E., WHITBY, J.L., The rationale and a computer evaluation of a gamma irradiation sterilization dose determination method for medical devices using a substerilization incremental dose sterility test protocol, J. Appl. Bacteriol. **57** (1984) 31–50.

[III–2] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, Sterilization of Health Care Products — Radiation Sterilization — Substantiation of 25kGy as a Sterilization Dose for Small or Infrequent Production Batches, Rep. ISO/TR 13409: 1996, ISO, Geneva (1996).

[III–3] ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION, Sterilization of Health Care Products — Radiation Sterilization — Substantiation of 25 kGy as Sterilization Dose — Method VD_{max}, Rep. TIR 27:2001, AAMI, Arlington, VA (2001).

TABLE III–1. MICROBIAL STANDARD DISTRIBUTION OF RESISTANCE [III–1]

D_{10} (kGy)	1.0	1.5	2.0	2.5	2.8	3.1	3.4	3.7	4.0	4.2
Per cent of samples	65.487	22.493	6.302	3.179	1.213	0.786	0.350	0.111	0.072	0.007

TABLE III–2. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A GIVEN STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDENS (cfu) HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Sample size (n)	SAL ($1/n$)	Bioburden															
		0.06	0.08	0.09	0.1	0.12	0.14	0.17	0.19	0.22	0.26	0.29	0.34	0.39	0.44	0.5	0.57
10	1/10								0.3	0.4	0.5	0.5	0.6	0.7	0.8	0.9	0.9
15	1/15						0.4	0.5	0.6	0.6	0.7	0.8	0.9	1.0	1.0	1.1	1.2
20	1/20				0.4	0.5	0.5	0.6	0.7	0.8	0.9	1.0	1.0	1.1	1.2	1.3	1.4
25	1/25		0.4	0.4	0.5	0.6	0.7	0.8	0.8	0.9	1.0	1.1	1.2	1.3	1.3	1.4	1.5
30	1/30		0.5	0.5	0.6	0.7	0.8	0.9	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.5	1.6
35	1/35	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.6	1.7
40	1/40	0.5	0.6	0.7	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.6	1.7	1.8
45	1/45	0.5	0.7	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.4	1.5	1.6	1.7	1.8	1.9
50	1/50	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
60	1/60	0.7	0.9	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.6	1.7	1.8	1.9	2.0	2.1
70	1/70	0.8	0.93	1.0	1.1	1.2	1.3	1.4	1.4	1.5	1.7	1.7	1.9	2.0	2.0	2.1	2.2
80	1/80	0.8	1.0	1.1	1.1	1.2	1.3	1.5	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3
90	1/90	0.9	1.1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4
100	1/100	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.1	2.3	2.4	2.5

TABLE III–3. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A GIVEN STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDENS (cfu) HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Sample size (n)	SAL ($1/n$)	Bioburden															
		0.65	0.73	0.83	0.93	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.6	3.0	3.2	4.0	4.4
10	1/10	1.0	1.1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.7	1.8	1.9	2.0	2.1	2.2	2.3
15	1/15	1.3	1.3	1.4	1.5	1.5	1.7	1.8	1.9	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6
20	1/20	1.4	1.5	1.6	1.7	1.7	1.9	2.0	2.1	2.2	2.2	2.3	2.4	2.5	2.6	2.8	2.9
25	1/25	1.6	1.7	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	3.0	3.0
30	1/30	1.7	1.8	1.9	2.0	2.0	2.1	2.3	2.4	2.5	2.5	2.6	2.7	2.9	2.9	3.1	3.2
35	1/35	1.8	1.9	2.0	2.1	2.1	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.3	3.4
40	1/40	1.9	2.0	2.1	2.2	2.2	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5
45	1/45	2.0	2.1	2.2	2.3	2.3	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.5	3.6
50	1/50	2.1	2.1	2.2	2.3	2.4	2.5	2.7	2.8	2.9	3.0	3.0	3.2	3.3	3.4	3.6	3.7
60	1/60	2.2	2.3	2.4	2.5	2.5	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5	3.5	3.8	3.9
70	1/70	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.2	3.3	3.3	3.5	3.6	3.7	3.9	4.0
80	1/80	2.4	2.5	2.6	2.7	2.8	2.9	3.1	3.2	3.3	3.4	3.5	3.6	3.8	3.8	4.0	4.1
90	1/90	2.5	2.6	2.7	2.8	2.9	3.0	3.2	3.3	3.4	3.5	3.6	3.7	3.9	3.9	4.1	4.2
100	1/100	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5	3.6	3.7	3.8	4.0	4.0	4.2	4.3

TABLE III–4. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A GIVEN STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDENS (cfu) HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Sample size (n)	SAL ($1/n$)	Bioburden													
		5.0	5.4	6.0	7.0	8.0	9.0	10	11	12	13	14	15	16	17
10	1/10	2.4	2.5	2.5	2.7	2.8	2.9	3.0	3.0	3.1	3.2	3.3	3.3	3.4	3.4
15	1/15	2.7	28	2.9	3.0	3.1	3.2	3.4	3.5	3.6	3.7	3.7	3.8	3.8	3.9
20	1/20	3.0	3.0	3.1	3.3	3.4	3.5	3.6	3.7	3.7	3.8	3.9	4.0	4.0	4.1
25	1/25	3.2	3.2	3.3	3.5	3.6	3.7	3.8	3.9	4.0	4.0	4.1	4.2	4.2	4.3
30	1/30	3.3	3.4	3.5	3.6	3.8	3.9	4.1	4.1	4.2	4.3	4.4	4.4	4.5	4.6
35	1/35	3.5	3.6	3.6	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.4	4.5	4.6	4.6
40	1/40	3.6	3.7	3.8	3.9	4.0	4.2	4.3	4.4	4.4	4.5	4.6	4.7	4.7	4.8
45	1/45	3.7	3.8	3.9	4.0	4.2	4.3	4.4	4.5	4.6	4.7	4.7	4.8	4.9	4.9
50	1/50	3.8	3.9	4.0	4.1	4.3	4.4	4.4	4.5	4.6	4.7	4.8	4.9	4.9	5.0
60	1/60	4.0	4.1	4.2	4.3	4.4	4.6	4.7	4.8	4.9	5.0	5.0	5.1	5.2	5.3
70	1/70	4.1	4.2	4.3	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.3	5.4
80	1/80	4.2	4.3	4.4	4.6	4.7	4.9	5.0	5.1	5.2	5.3	5.3	5.4	5.5	5.6
90	1/90	4.4	4.5	4.6	4.7	4.8	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.6	5.7
100	1/100	4.5	4.6	4.7	4.8	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.7	5.8

TABLE III–5. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A GIVEN STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDENS (cfu) HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Sample size (n)	SAL ($1/n$)	Bioburden														
		18	19	20	25	30	35	40	45	50	55	60	65	70	75	80
10	1/10	3.5	3.5	3.6	3.8	4.0	4.1	4.3	4.4	4.5	4.6	4.7	4.8	4.8	4.9	5.0
15	1/15	4.0	4.0	4.1	4.3	4.5	4.6	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.4	5.5
20	1/20	4.1	4.2	4.2	4.5	4.6	4.8	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.6	5.7
25	1/25	4.3	4.4	4.5	4.7	4.9	5.0	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	5.9
30	1/30	4.6	4.7	4.7	5.0	5.2	5.3	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.2
35	1/35	4.7	4.7	4.8	5.0	5.2	5.4	5.6	5.7	5.9	6.0	6.1	6.2	6.2	6.3	6.4
40	1/40	4.9	4.9	5.0	5.2	5.4	5.6	5.7	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6
45	1/45	5.0	5.1	5.1	5.4	5.6	5.7	5.9	6.0	6.1	6.3	6.4	6.4	6.5	6.6	6.7
50	1/50	5.0	5.1	5.1	5.4	5.6	5.7	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7
60	1/60	5.3	5.4	5.4	5.7	5.9	6.1	6.2	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.0
70	1/70	5.5	5.5	5.6	5.9	6.1	6.2	6.4	6.5	6.7	6.8	6.9	7.0	7.1	7.2	7.2
80	1/80	5.6	5.7	5.7	6.0	6.2	6.4	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4
90	1/90	5.8	5.8	5.9	6.1	6.3	6.5	6.7	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.5
100	1/100	5.9	5.9	6.0	6.3	6.5	6.6	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7

TABLE III–6. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A GIVEN STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDENS (cfu) HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Sample size (n)	SAL ($1/n$)	Bioburden											
		85	90	95	100	150	200	250	300	350	400	450	500
10	1/10	5.0	5.1	5.2	5.2	5.7	6.0	6.2	6.5	6.6	6.7	6.9	7.1
15	1/15	5.6	5.6	5.7	5.8	6.2	6.6	6.8	7.0	7.2	7.4	7.5	7.7
20	1/20	5.8	5.8	5.9	5.9	6.4	6.8	7.0	7.2	7.4	7.6	7.7	7.8
25	1/25	6.0	6.1	6.1	6.2	6.7	7.0	7.3	7.5	7.7	7.9	8.0	8.1
30	1/30	6.3	6.4	6.4	6.5	7.0	7.3	7.6	7.8	8.0	8.2	8.3	8.5
35	1/35	6.5	6.5	6.6	6.7	7.1	7.5	7.8	8.0	8.2	8.4	8.5	8.7
40	1/40	6.6	6.7	6.8	6.8	7.3	7.7	7.9	8.2	8.4	8.5	8.7	8.8
45	1/45	6.8	6.8	6.9	7.0	7.5	7.8	8.1	8.3	8.5	8.7	8.9	9.0
50	1/50	6.9	7.0	7.0	7.1	7.6	7.9	8.2	8.5	8.7	8.8	9.0	9.1
60	1/60	7.1	7.2	7.3	7.3	7.8	8.2	8.5	8.7	8.9	9.1	9.2	9.4
70	1/70	7.3	7.4	7.4	7.5	8.0	8.4	8.7	8.9	9.1	9.3	9.4	9.6
80	1/80	7.5	7.5	7.6	7.7	8.2	8.5	8.8	9.1	9.3	9.4	9.6	9.7
90	1/90	7.6	7.7	7.8	7.8	8.3	8.7	9.0	9.2	9.4	9.6	9.8	9.9
100	1/100	7.7	7.8	7.9	7.9	8.5	8.8	9.1	9.4	9.5	9.7	9.9	10.0

TABLE III–7. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A GIVEN STERILITY ASSURANCE LEVEL FOR DIFFERENT BIOBURDENS (cfu) HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Sample size (n)	SAL ($1/n$)	Bioburden									
		550	600	650	700	750	800	850	900	950	1000
10	1/10	7.2	7.3	7.4	7.5	7.6	7.6	7.7	7.8	7.9	7.9
15	1/15	7.8	7.9	8.0	8.1	8.2	8.2	8.3	8.4	8.5	8.5
20	1/20	8.0	8.1	8.2	8.3	8.4	8.5	8.5	8.6	8.7	8.7
25	1/25	8.2	8.4	8.5	8.5	8.6	8.7	8.8	8.9	8.9	9.0
30	1/30	8.6	8.7	8.8	8.9	9.0	9.0	9.1	9.2	9.3	9.3
35	1/35	8.8	8.9	9.0	9.1	9.2	9.3	9.3	9.4	9.5	9.6
40	1/40	9.0	9.1	9.2	9.3	9.4	9.4	9.5	9.6	9.7	9.7
45	1/45	9.1	9.2	9.3	9.4	9.5	9.6	9.7	9.8	9.8	9.9
50	1/50	9.2	9.3	9.5	9.6	9.7	9.7	9.8	9.9	10.0	10.0
60	1/60	9.5	9.6	9.7	9.8	9.9	10.0	10.1	10.1	10.2	10.3
70	1/70	9.7	9.8	9.9	10.0	10.1	10.2	10.3	10.3	10.4	10.5
80	1/80	9.9	10.0	10.1	10.2	10.3	10.4	10.4	10.5	10.6	10.7
90	1/90	10.0	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	10.8
100	1/100	10.2	10.3	10.4	10.5	10.6	10.6	10.7	10.8	10.9	11.0

TABLE III–8. RADIATION DOSE (kGy) REQUIRED TO ACHIEVE A STERILITY ASSURANCE LEVEL OF 10^{-6} FOR DIFFERENT BIOBURDENS HAVING A STANDARD DISTRIBUTION OF RESISTANCE

Bioburden	Dose	Bioburden	Dose	Bioburden	Dose
0.06	10.4	2.0	15.2	30	19.3
0.08	10.6	2.2	15.3	40	19.7
0.09	10.8	2.6	15.5	50	20.1
0.10	11.0	3.0	15.8	60	20.3
0.12	11.3	3.2	16.0	70	20.6
0.14	11.5	4.0	16.2	80	20.8
0.17	11.7	4.4	16.3	90	21.0
0.19	11.9	5.0	16.5	100	21.1
0.22	12.1	5.4	16.6	150	21.8
0.26	12.3	6.0	16.8	200	22.2
0.29	12.5	7.0	17.0	250	22.6
0.34	12.7	8.0	17.2	300	22.9
0.39	12.9	8.8	17.3	350	23.1
0.44	13.1	9.0	17.4	400	23.3
0.50	13.3	10	17.6	450	23.5
0.57	13.5	11	17.7	500	23.7
0.65	13.6	12	17.9	550	23.8
0.73	13.8	13	18.0	600	24.0
0.83	14.0	14	18.1	650	24.1
0.93	14.2	15	18.2	700	24.2
1.0	14.2	16	18.3	750	24.3
1.2	14.3	17	18.4	800	24.4
1.4	14.6	18	18.5	850	24.5
1.6	14.8	19	18.6	900	24.6
1.8	14.9	20	18.7	950	24.7
				1000	24.8

TABLE III–9. *I* AND *S* FOR CALCULATION OF THE VERIFICATION DOSE FOR A GIVEN TEST SAMPLE SIZE AND BIOBURDEN LEVEL [III–2]

Test sample size	Bioburden 1–10		Bioburden 11–100		Bioburden 101–1000	
	I	*S*	*I*	*S*	*I*	*S*
10	1.25	1.65	0.67	2.23	–0.26	2.71
20	1.71	1.82	1.14	2.41	0.35	2.81
30	2.00	1.93	1.46	2.49	0.71	2.87
40	2.21	2.01	1.69	2.55	1.00	2.90
50	2.38	2.07	1.88	2.59	1.21	2.93
60	2.52	2.12	2.03	2.63	1.40	2.95
70	2.65	2.16	2.16	2.66	1.55	2.97
80	2.76	2.19	2.30	2.67	1.67	2.99
90	2.86	2.22	2.39	2.70	1.80	3.00

Note: Verification dose at a given SAL = $I + (S \times \log(\text{average SIP bioburden}))$;
I = intercept; S = slope.

TABLE III–10. VERIFICATION DOSES AND DOSE REDUCTION FACTORS FOR A STERILITY ASSURANCE LEVEL OF 10^{-1} USING METHOD C
(extracted from Ref. [III–3])

Bioburden	VD (kGy)	DRF	Bioburden	VD (kGy)	DRF	Bioburden	VD (kGy)	DRF
1	4.2	4.17	40	8.6	3.29	280	8.6	2.49
2	5.2	3.97	45	8.7	3.27	300	8.6	2.46
3	5.7	3.86	50	8.8	3.25	325	8.5	2.43
4	6.1	3.79	55	8.9	3.23	350	8.5	2.40
5	6.3	3.73	60	8.9	3.21	375	8.5	2.37
6	6.6	3.69	65	9.0	3.20	400	8.4	2.34
7	6.7	3.65	70	9.1	3.19	425	8.4	2.32
8	6.9	3.62	75	9.1	3.17	450	8.4	2.30
9	7.0	3.59	80	9.2	3.15	475	8.4	2.28
10	7.1	3.57	85	9.1	3.11	500	8.4	2.26
11	7.2	3.55	90	9.1	3.08	525	8.3	2.24
12	7.3	3.53	95	9.1	3.05	550	8.3	2.22
13	7.4	3.51	100	9.0	3.01	575	8.3	2.21
14	7.5	3.50	110	9.0	2.96	600	8.3	2.19
15	7.6	3.48	120	9.0	2.91	650	8.3	2.15
16	7.6	3.47	130	8.9	2.86	700	8.2	2.14
17	7.7	3.46	140	8.9	2.83	750	8.2	2.12
18	7.8	3.45	150	8.9	2.79	800	8.2	2.09
19	7.8	3.43	160	8.8	2.76	850	8.2	2.07
20	7.9	3.42	170	8.8	2.72	900	8.1	2.05
22	8.0	3.40	180	8.8	2.69	950	8.1	2.04
24	8.1	3.39	190	8.7	2.67	1000	8.1	2.02
26	8.1	3.37	200	8.7	2.64			
28	8.2	3.36	220	8.7	2.60			
30	8.3	3.34	240	8.6	2.56			
35	8.4	3.31	260	8.6	2.52			

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CONTRIBUTORS TO DRAFTING AND REVIEW

Abdurrahman, A.	National Nuclear Energy Agency, Indonesia
Anderson, M.A.	Musculoskeletal Transplant Foundation, United States of America
Benaim, F.	Fundación del Quemado “F. Benaim”, Argentina
Doppelt, S.	Cambridge Hospital, United States of America
Dosekova, E.	International Atomic Energy Agency
Hendry, J.	International Atomic Energy Agency
Kairiyama, E.	National Atomic Energy Commission, Argentina
Koller, J.	Ruzinov Central Hospital, Slovakia
Manyalich, M.	Universidad de Barcelona, Spain
Mohamad, H.	Hospital Kota Bharu, Malaysia
Morales, J.	International Atomic Energy Agency
Nather, A.A.M.	National University Hospital, Singapore
Parsons, B.J.	North East Wales Institute of Higher Education, United Kingdom
Phillips, G.O.	Research Transfer Ltd, United Kingdom
Salai, M.	Chaim Sheba Medical Centre, Israel
Von Versen, R.	Deutsches Institut für Zell- und Gewebeersatz, Germany

This code of practice contains internationally agreed recommendations and guidelines for the safe use of ionizing radiation as a sterilization procedure for tissue allografts. The recommendations were produced by the IAEA with the help and approval of the main professional associations of tissue banks in Europe, Latin America and the USA and by experts in all aspects of radiosterilization and transplantation procedures. The code of practice covers the recommended qualifications of the tissue bank facilities, the tissue donors, the tissue processing and preservation procedures, and the maintenance of validation of the pre-sterilization and sterilization processes. Also covered are the quality, safety and clinical application of the tissue allografts, documentation and certification procedures, management and control issues, establishing a sterilization dose and worked examples.

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