INTERNATIONAL STANDARD

ISO 14160

Third edition
2020-09

Sterilization of health care products — Liquid chemical sterilizing agents for single-use medical devices utilizing animal tissues and their derivatives — Requirements for characterization, development, validation and routine control of a sterilization process for medical devices

Stérilisation des produits de santé — Agents stérilisants chimiques liquides pour dispositifs médicaux non réutilisables utilisant des tissus animaux et leurs dérivés — Exigences pour la caractérisation, le développement, la validation et le contrôle de routine d'un procédé de stérilisation de dispositifs médicaux

Reference number
ISO 14160:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 204, *Sterilization of medical devices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 14160:2011), which has been technically revised.

The main changes compared to the previous edition are as follows:

— aligned definitions with those used in other standards for development, validation and routine control of sterilization processes and added new definitions;

— incorporated defined terms consistently throughout the document;

— updated cross-references;

— revised informative Annex A to follow the order of the normative body of the standard;

— added clarification to normative Annex B in regard to applying the overkill approach.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

A sterile medical device is one that is free of viable microorganisms. International standards which specify requirements for validation and routine control of sterilization processes require, when it is necessary to supply a sterile medical device, that adventitious microbiological contamination of a medical device prior to sterilization be minimized. Even so, medical devices produced under standard manufacturing conditions in accordance with the requirements for quality management systems (see for example, ISO 13485) have microorganisms on them prior to sterilization, albeit in low numbers. The purpose of sterilization is to inactivate the microbiological contaminants and thereby transform the non-sterile medical devices into sterile ones.

The kinetics of inactivation of a pure culture of microorganisms by physical and/or chemical agents used to sterilize medical devices can generally best be described by an exponential relationship between the numbers of microorganisms surviving and the extent of treatment with the sterilizing agent; inevitably this means that there is always a finite probability that a microorganism survives regardless of the extent of treatment applied. For a given treatment, the probability of survival is determined by the number and resistance of microorganisms and by the environment in which the organisms exist during treatment. It follows that the sterility of any one medical device in a population of items subjected to sterilization processing cannot be guaranteed and the sterility of a processed population is defined in terms of the probability of there being a viable microorganism present on a medical device.

Attention also has to be given to a number of factors, including the microbiological status (bioburden) of incoming raw materials and/or components and their subsequent storage, and to the control of the environment in which the product is manufactured, assembled and packaged (see also ISO 13485).

Requirements for quality management systems for medical device production are given in ISO 13485. The standards for quality management systems recognize that for certain processes used in manufacturing, the effectiveness of the process cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of such a process. For this reason, sterilization processes are validated for use, the performance of the sterilization process is monitored routinely, and the equipment is maintained.

Animal tissues and their derivatives are used as constituents of certain medical devices to provide performance characteristics that present advantages over the characteristics provided by non-animal-based materials. The range and quantities of materials of animal origin in medical devices vary; such materials can comprise a major part of the device, can be a product coating or impregnation, or can be used in the manufacturing process for the medical device.

This document describes requirements that, if met, will provide a liquid chemical sterilization process that has appropriate microbicidal activity for single-use medical devices containing materials of animal origin or their derivatives. The sterilizing agents used most frequently for medical devices are moist heat, dry heat, irradiation and ethylene oxide. While some devices containing animal tissues can be compatible with these commonly applied methods of sterilization (historically, for example, catgut sutures have been sterilized by irradiation), other devices, such as biological heart valves or tissue patches, are not compatible with conventional sterilization processes. It has been recognized that other sterilizing agents could have to be used in these exceptional circumstances. Liquid chemical sterilization is normally chosen over other sterilization processes in order that the medical devices present the desired physical properties of the tissue after sterilization. Sterilization by liquid chemicals of medical devices made in whole or in part from tissues of animal origin represents a special case in terms of establishing an effective sterilization process. In common with the other sterilization methods, the efficacy of a liquid chemical sterilization process needs to be demonstrated and recorded before it is adopted for routine use.

Liquid chemical sterilization requires determination of types of microorganisms comprising the bioburden and their resistance to the sterilization process in order to establish the appropriate reference microorganism, whether that be a recognized biological indicator or an isolate from the bioburden. Compliance with the requirements of this document ensures that the microbicidal activity of the liquid chemical sterilization process is both reliable and reproducible so that predictions can be made, with reasonable confidence, that there is a low level of probability of there being a viable

microorganism present on a product after sterilization. Specification of this probability is a matter for regulatory authorities and can vary among regions or countries (see for example, EN 556-1 and ANSI/AAMI ST67).

Exposure to a properly validated, accurately controlled sterilization process is not the only factor associated with the provision of reliable assurance that the product is sterile and, in this regard, suitable for its intended use. Attention is therefore given to a number of considerations including:

a) the source and harvesting conditions of the tissue;

b) the microbiological status of incoming raw materials or components, or both;

c) the routine control of any cleaning and disinfection procedures used on the product;

d) the control of the environment in which the product is manufactured, assembled and packaged;

e) the control of equipment and processes;

f) the control of personnel and their hygiene;

g) the manner and materials in which the product is packaged; and

h) the conditions under which product is stored.

Sterilization of health care products — Liquid chemical sterilizing agents for single-use medical devices utilizing animal tissues and their derivatives — Requirements for characterization, development, validation and routine control of a sterilization process for medical devices

1 Scope

This document specifies requirements for the characterization of a liquid chemical sterilizing agent and for the development, validation, process control and monitoring of sterilization by liquid chemical sterilizing agents of single-use medical devices comprising, in whole or in part, materials of animal origin.

This document covers the control of risks arising from contamination with bacteria and fungi by application of a liquid chemical sterilization process. Risks associated with other microorganisms can be assessed using other methods (see NOTE 1).

This document is not applicable to material of human origin.

This document does not describe methods for the validation of the inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents (see NOTE 2 and NOTE 3).

This document does not describe methods for validation of the inactivation or elimination of protozoa and parasites.

The requirements for validation and routine control described in this document are only applicable to the defined sterilization process of a medical device, which is performed after the manufacturing process, and do not take account of the lethal effects of other bioburden reduction steps (see NOTE 4).

This document does not specify tests to establish the effects of any chosen sterilization process upon the fitness for use of the medical device (see NOTE 5).

This document does not cover the level of residual sterilizing agent within medical devices (see NOTE 6).

Guidance for the characterization of a liquid chemical sterilizing agent and for the development, validation, process control and monitoring of sterilization by liquid chemical sterilizing agents of single-use medical devices comprising, in whole or in part, materials of animal origin is provided in informative Annex A.

NOTE 1 The prior application of risk management principles to medical devices utilizing animal tissues, as described in ISO 22442-1 is important. ISO 18362 provides information on control of microbial risks during processing of cell-based health-care products.

NOTE 2 Liquid chemical sterilizing agents traditionally employed to sterilize animal tissues in medical devices might not be effective in inactivating the causative agents of TSE such as bovine spongiform encephalopathy (BSE), or scrapie. Satisfactory validation in accordance with this document does not necessarily demonstrate inactivation of infective agents of this type. Risk controls related to sourcing, collection and handling of animal materials are described in ISO 22442-2.

NOTE 3 The validation of the inactivation, elimination, or elimination and inactivation of viruses and TSE agents is described in ISO 22442-3.

NOTE 4 Manufacturing processes for medical devices containing animal tissues frequently include exposure to chemical agents which can significantly reduce the bioburden on the medical device. Following the manufacturing process, a medical device is exposed to a specified sterilization process.

NOTE 5 Such testing is a crucial part of the design and development of a medical device.

NOTE 6 ISO 10993-17 specifies a method to establish allowable limits for residues of sterilizing agents.

NOTE 7 Standards for quality management systems (see ISO 13485) can be used in the control of all stages of manufacture including the sterilization process.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

ISO 10993-17, *Biological evaluation of medical devices — Part 17: Establishment of allowable limits for leachable substances*

ISO 11737-1, *Sterilization of health care products — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

ISO 13408 (all parts), *Aseptic processing of health care products*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
batch
defined quantity of product, intended or purported to be uniform in character and quality produced during a defined cycle of manufacture

[SOURCE: ISO 11139:2018, 3.21]

3.2
bioburden
population of viable microorganisms on or in product and/or sterile barrier system

[SOURCE: ISO 11139:2018, 3.23]

3.3
carrier
<biological indicator> supporting material on or in which test microorganisms are deposited

[SOURCE: ISO 11139:2018, 3.33]

3.4
***D* value**
D_{10} value
time or dose required under stated conditions to achieve inactivation of 90 % of a population of the test microorganisms

[SOURCE: ISO 11139:2018, 3.75]

3.5
holding time
period during which process parameters are maintained, within their specified tolerances

[SOURCE: ISO 11139:2018, 3.133]

3.6
inactivation curve
graphical representation of decrease in viability of a population of microorganisms with increasing exposure to a microbicidal agent under stated conditions

[SOURCE: ISO 11139:2018, 3.137]

3.7
inoculated carrier
supporting material on or in which a specified number of viable test microorganisms has been deposited

[SOURCE: ISO 11139:2018, 3.144]

3.8
installation qualification
IQ
process of establishing by objective evidence that all key aspects of the process equipment and ancillary system installation comply with the approved specification

[SOURCE: ISO 11139:2018, 3.220.2]

3.9
liquid chemical sterilizing agent
liquid chemical entity, or combination of entities, having sufficient microbicidal activity to achieve *sterility* (3.21) under defined conditions

[SOURCE: ISO 11139:2018, 3.288, modified— "liquid chemical" added to term and in definition "physical or chemical entity is "replaced by "liquid chemical entity "]

3.10
medical device
instrument, apparatus, implement, machine, appliance, implant, reagent for in vitro use, or software, material, or other similar related article, intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific medical purpose(s) of:

— diagnosis, prevention, monitoring, treatment or alleviation of disease;

— diagnosis, monitoring, treatment, alleviation of or compensation for an injury;

— investigation, replacement, modification or support of the anatomy or of a physiological process;

— supporting or sustaining life;

— control of conception;

— disinfection of medical devices;

— providing information by means of in vitro examination of specimens derived from the human body;

and does not achieve its primary intended action by pharmacological, immunological or metabolic means, but which may be assisted in its intended function by such means

Note 1 to entry: Products which may be considered to be medical devices in some jurisdictions but not in others include:

— items specifically intended for cleaning, packaging, or sterilization of medical devices

— disinfection substances;

— aids for persons with disabilities;

— devices incorporating animal and/or human tissues;

— devices for in vitro fertilization or assisted reproduction technologies.

[SOURCE: ISO 11139:2018, 3.166]

3.11
microbial inactivation
inactivation
loss of ability of microorganisms to grow and/or multiply

[SOURCE: ISO 11139:2018, 3.172, modified — "inactivation" added as admitted term]

3.12
operational qualification
OQ
process of obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures

[SOURCE: ISO 11139:2018, 3.220.3]

3.13
parametric release
declaration that product is *sterile* (3.23), based on records demonstrating that the sterilization process variables were delivered within specified tolerances

[SOURCE: ISO 11139:2018, 3.193]

3.14
performance qualification
PQ
process of establishing by objective evidence that the process, under anticipated conditions, consistently produces a product which meets all predetermined requirements

[SOURCE: ISO 11139:2018, 3.220.4]

3.15
product family
group or subgroup of product characterized by similar attributes determined to be equivalent for evaluation and processing purposes

[SOURCE: ISO 11139:2018, 3.218]

3.16
reference microorganism
microbial strain obtained from a recognized culture collection

[SOURCE: ISO 11139:2018, 3.228]

3.17
requalification
repetition of part or all of validation for the purpose of confirming the continued acceptability of a specified process

[SOURCE: ISO 11139:2018, 3.220.5]

3.18
specify
stipulate in detail within an approved document

[SOURCE: ISO 11139:2018, 3.259]

3.19
sterile
free from viable microorganisms

[SOURCE: ISO 11139:2018, 3.271]

3.20
sterile barrier system
SBS
minimum package that minimizes the risk of ingress of microorganisms and allows aseptic presentation of the *sterile* (3.19) contents at the point of use

[SOURCE: ISO 11139:2018, 3.272]

3.21
sterility
state of being free from viable microorganisms

Note 1 to entry: In practice, no such absolute statement regarding the absence of microorganisms can be proven

[SOURCE: ISO 11139:2018, 3.274]

3.22
sterility assurance level
SAL
probability of a single viable microorganism occurring on an item after *sterilization* (3.23)

Note 1 to entry: It is expressed as the negative exponent to the base 10.

[SOURCE: ISO 11139:2018, 3.275]

3.23
sterilization
validated process used to render a product free from viable microorganisms

Note 1 to entry: In a sterilization process, the nature of microbial inactivation is exponential and thus the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero.

[SOURCE: ISO 11139:2018, 3.277]

3.24
storage solution
liquid in which a medical device in its final form is presented for use

[SOURCE: ISO 11139:2018, 3.290]

3.25
surrogate product
item designed to represent product in process simulations and which is comparable with the actual product

[SOURCE: ISO 11139:2018, 3.291]

3.26
test for sterility
technical operation specified in a pharmacopoeia performed on product following an aseptic process or exposure to a *sterilization* (3.23) process

[SOURCE: ISO 11139:2018, 3.298]

3.27
tissue
organization of cells, cells and extra-cellular constituents, or extra-cellular constituents

[SOURCE: ISO 11139:2018, 3.303]

3.28
validation
confirmation process, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The objective evidence needed for a validation is the result of a test or other form of determination such as performing alternative calculations or reviewing documents.

Note 2 to entry: The word "validated" is used to designate the corresponding status.

Note 3 to entry: The use conditions for validation can be real or simulated.

Note 4 to entry: For sterilization by liquid chemical sterilizing agents, validation is considered as a total programme, which consists of installation qualification, operational qualification and performance qualification.

[SOURCE: ISO 11139:2018, 3.313, modified — Note 4 to entry has been added]

4 General

4.1 The development, validation and routine control of a sterilization process is a critical element in product realization of health care product. To ensure the consistent implementation of the requirements specified in this document, the necessary processes need to be established, implemented and maintained. Processes of particular importance in relation to the development, validation and routine control of a sterilization process include but are not limited to:

— control of documentation, including records,

— assignment of management responsibility,

— provision of adequate resources, including competent human resources and infrastructure,

— control of product provided by external parties,

— identification and traceability of product throughout the process, and

— control of non-conforming product.

NOTE ISO 13485 covers all stages of the lifecycle of medical devices in the context of quality management systems for regulatory purposes. National and/or regional regulatory requirements for the provision of health care product can require the implementation of a full quality management system and the assessment of that system by a recognized conformity assessment body.

4.2 A process shall be specified for the calibration of all equipment, including instrumentation for test purposes, used in meeting the requirements of this document.

5 Sterilizing agent characterization

5.1 General

The purpose of this activity is to define the liquid chemical sterilizing agent, demonstrate its microbicidal effectiveness, identify the factors which influence microbicidal effectiveness, assess the effects that exposure to the liquid chemical sterilizing agent has on materials, and identify requirements for the safety of personnel and protection of the environment.

NOTE In some cases the storage solution is used as the liquid chemical sterilizing agent, in which case the requirements of Clause 5 apply to the storage solution, the medical device, and the internal surfaces of the sterile barrier system.

5.2 Sterilizing agent

5.2.1 The liquid chemical sterilizing agent shall be specified. This specification shall include, if appropriate:

a) the formulation of the liquid chemical sterilizing agent, including concentration(s) of the active agent(s) and pH;

b) an expiration date;

c) a statement that the sterilizing agent shall not be reused;

d) the storage conditions.

The specification for the liquid chemical sterilizing agent should take into account possible contaminants that could affect the suitability of the processed animal material for its intended use.

5.2.2 The means of ensuring that the liquid chemical sterilizing agent is free from viable microorganisms before use shall be specified.

5.3 Microbicidal effectiveness

5.3.1 Microbicidal effectiveness studies shall

a) demonstrate the lethal action of the liquid chemical sterilizing agent against a range of representative microorganisms,

NOTE 1 If a liquid chemical sterilizing agent is commercially available, labelled for this intended use and has applicable regulatory approval, the microbicidal effectiveness can be demonstrated by the data on a range of representative microorganisms generated by the manufacturer of the liquid chemical sterilizing agent, with verification by the manufacturer of the medical device.

NOTE 2 Guidance on microorganism selection is included in Table A.1.

b) identify the process variables that affect the lethal action of the liquid chemical sterilizing agent, e.g. time, temperature, liquid chemical sterilizing agent concentration and pH (potential interactions of process variables should be considered),

c) assess those factors that can adversely affect the delivery, or distribution, or both, of the liquid chemical sterilizing agent, and those that can influence its effectiveness [i.e. the liquid chemical sterilizing agent(s) should be able to reach all areas since microorganisms could be inside cell/tissue structures], and

d) assess the microbicidal effectiveness of the liquid chemical sterilizing agent at the tolerance limits for the combination of process variables that results in the lowest microbicidal activity.

5.3.2 The microbicidal effectiveness studies shall include a screening test to identify microorganisms with a high resistance to the process. This shall include organisms from the product bioburden and the environment, as well as a reference organism(s) known to be innately resistant to the liquid chemical sterilizing agent.

5.4 Effects on materials

5.4.1 The effects of exposure to the liquid chemical sterilizing agent on the physical or chemical properties of component materials of the medical devices, and on their suitability for use, shall be assessed. The materials used in the assessment should be selected on the basis of their likely use in products to be treated with the liquid chemical sterilizing agent.

5.4.2 If the product is to be exposed repeatedly to the liquid chemical sterilizing agent, the effects of such multiple exposures on properties of component materials using the combination of process parameters likely to maximize material effects shall be evaluated.

5.4.3 The materials tested and the outcomes of all tests shall be recorded together with the criteria against which the properties of materials were assessed before and after exposure to the liquid chemical sterilizing agent.

5.5 Safety and the environment

5.5.1 Either a safety data sheet (SDS) or analogous safety information shall be specified for the liquid chemical sterilizing agent. This SDS may be provided by a supplier for a chemical agent or be prepared as a prelude to experimental studies on the liquid chemical sterilizing agent.

5.5.2 The potential impact on the environment of any substance which could be released, either deliberately or accidentally, during or following use of the liquid chemical sterilizing agent, shall be assessed and measures established for the control of the substance(s). This assessment, including the potential impact (if any) and the measures for control (if identified), shall be recorded.

6 Process and equipment characterization

6.1 General

The purpose of this activity is to define the entire sterilization process and the equipment necessary to deliver the sterilization process safely and reproducibly.

6.2 Process characterization

6.2.1 The process parameters, together with their tolerances, shall be specified. These tolerances shall:

a) be based upon knowledge of the combination of process parameters yielding minimum effectiveness; and

b) yield acceptable product.

6.2.2 Means of monitoring and controlling the process variables shall be specified.

6.2.3 Any treatment of the product that is required prior to exposure to the sterilization process to ensure effectiveness of the process shall be specified.

6.2.4 Any treatment of the product that is required following exposure to the liquid chemical sterilizing agent to ensure safety of the product shall be specified as part of the sterilization process.

6.3 Equipment characterization

6.3.1 The equipment to deliver the process within the tolerances stipulated for the process parameters and in a safe manner shall be specified.

6.3.2 The specification shall include, but is not limited to:

a) a description of the equipment and necessary ancillary items, including materials of construction,

b) the means by which the specified liquid chemical sterilizing agent (see 5.2) is provided, including any additives or precursors necessary for its delivery,

c) a description of the instrumentation for monitoring and controlling the sterilization process, including sensor characteristics and locations, indicating and recording instruments,

d) any fault recognition by the sterilizing equipment, if appropriate,

e) any safety features, including those for personnel and environmental protection,

f) the installation requirements, including for the control of emissions, if appropriate, and

g) the conditions for storage of the liquid chemical sterilizing agent within the equipment to ensure that its quality and composition remain within specifications, if applicable.

6.3.3 Software that is used to control or to monitor the process shall be prepared and validated in accordance with a quality management system that provides documented evidence that the software meets its design specification.

NOTE For guidance, see ISO/IEC/IEEE 90003.

6.3.4 Means shall be provided to ensure that failure in a control function does not lead to a failure in recording of process parameters such that an ineffective process appears effective. This may be achieved either by the use of independent systems for control and monitoring, or by a crosscheck between control and monitoring which identifies discrepancies and indicates a fault.

7 Product definition

7.1 The purpose of this activity is to define the product to be sterilized by the liquid chemical sterilizing agent, including the microbiological quality of the product prior to sterilization and the manner in which the product is presented for sterilization.

7.2 Product to be sterilized (including dimensions) and its condition shall be specified. This shall include ancillary components and packaging, if applicable, and shall take into account the bioburden and quantity and types of tissue, and organic and inorganic contamination.

7.3 Product may be assigned to a product family. The criteria for assigning a product to a product family shall be specified and shall include consideration of bioburden. A demonstration of equivalence to previously validated product, package or loading pattern shall be considered to meet this requirement. Any demonstration of equivalence shall be documented.

7.4 Bioburden of the product as it is presented for sterilization shall be determined in accordance with ISO 11737-1.

NOTE The intention is that the bioburden be stable and low, given the nature of the raw materials, product and manufacturing procedures prior to sterilization.

7.5 Product and packaging shall be designed to allow contact with the liquid chemical sterilizing agent. The location within the product at which sterilization is most difficult to achieve shall be identified.

7.6 It shall have been demonstrated and documented that the sterilization process does not adversely affect the fitness for use of the product or its packaging. If re-sterilization is to be permitted, the effects of such processing shall be evaluated and documented.

7.7 The biological safety, in accordance with ISO 10993-1, and fitness for use of the product following exposure to the sterilization process shall be established. A risk assessment shall be conducted to identify and specify limits for process residuals in product, in accordance with ISO 10993-17.

7.8 Where the product is supplied in a solution of the liquid chemical sterilizing agent or storage solution, instructions for use of the product shall be provided to specify means to reduce to acceptable limits the residual liquid chemical sterilizing agents or storage solution from the product. Allowable limits shall be established in accordance with ISO 10993-17.

8 Process definition

8.1 Purpose

The purpose of this activity is to obtain a detailed specification for the sterilization process to be applied to the defined product (see 7.2), without compromising the safety, quality and performance of that product. The sterilization process shall be documented. Process definition may be conducted in the production chamber or in a developmental chamber.

8.2 Determination of the inactivation kinetics

8.2.1 The sterilization process applicable to the defined product shall be established. This shall be achieved by the determination of inactivation kinetics (see Annex B), and hence establishment of the process parameters. An empirical mathematical relationship defining the microbial inactivation kinetics of identified resistant microorganisms shall be established, and it shall be confirmed that the probability of a microorganism surviving exposure to a defined treatment can be predicted.

NOTE If a liquid chemical sterilizing agent is commercially available, labelled for this intended use and has applicable regulatory approval, the determination of the inactivation kinetics can be demonstrated by the data generated by the manufacturer of the liquid chemical sterilizing agent, with verification by the manufacturer of the medical device.

8.2.2 Inactivation kinetics shall be determined by the construction of inactivation curves of the number of surviving microorganisms plotted on a $\log_{10}$ scale against holding time for the microorganisms identified during the microbicidal effectiveness studies (see 5.3) as having a high resistance to the process. If it appears from the microbicidal effectiveness study that the resistance to the liquid chemical sterilizing agent of an isolate from the bioburden or manufacturing environment approaches or exceeds the resistance of the reference microorganism, then inactivation curves shall be constructed for both the bioburden or environmental isolates and the reference microorganism. If no isolates have a resistance to the liquid chemical sterilizing agent approaching that of the reference microorganism, then an inactivation curve shall be constructed only for the reference microorganism.

8.2.3 At minimum, inactivation kinetics shall be determined for that combination of process parameters (e.g. liquid chemical sterilizing agent concentration, temperature and pH) that has been identified as producing the lowest lethality during the liquid chemical sterilizing agent characterization. The rationale for the selection of these process parameters shall be documented.

8.2.4 The inactivation curve shall include a minimum of five points covering at least a thousandfold reduction in numbers. Microorganisms shall be presented to the process on carrier material(s)

representative of the medical device. Representative microorganisms from the pre-sterilization bioburden should be induced to grow on the tissue carriers, if possible (see Annex B).

If the product does not allow the above-mentioned procedure, the MPN (most probable number) method can be used. The MPN approach, if used, shall be rationalized and documented.

The *D* value(s) of the microorganism(s) identified in 8.2.2 based on the microbicidal effectiveness studies (see 5.3.1) shall be determined. The calculation of *D* value is only possible if the inactivation curve (the plot of number of survivors on a logarithmic scale against time of exposure) is linear. Where deviations from linearity occur, it can become difficult to predict a satisfactory sterilization process. Such deviations should be further investigated to better characterize the inactivation kinetics.

8.2.5 Process definition shall be performed in accordance with either B.1 or B.2. For a linear process, the sterilization holding time shall not be less than Formula (1).

$$D[6 + \log_{10}(100 + B)] \quad (1)$$

where

D is the *D* value of the most resistant microorganism identified above (see 8.2.4);

B is the value of the bioburden determined using ISO 11737-1.

If the overkill approach is used in microbiological performance qualification (MPQ), the sterilization holding time specified for the process shall not be less than either twice the time at which no survivors are recovered from a population of at least 10^6 reference microorganisms or $D \times 12$, where *D* is the *D* value of the most resistant organism identified above.

Construction of an inactivation curve is required irrespective of the method used.

NOTE The extended treatment specified by this clause provides a probability of at least 1×10^{-6} of microorganisms surviving treatment. EN 556-1 specifies this is a requirement for terminally-sterilized devices labelled sterile.

8.2.6 Maximum holding time of product to the liquid chemical sterilizing agent shall be specified.

8.3 Method for neutralization

The method for neutralization of the liquid chemical sterilizing agent prior to culturing for recovery of survivors shall be validated. The method shall not in itself adversely influence the ability to interpret the results.

8.4 Safety quality and performance

It shall be demonstrated that the product meets its specified requirements for safety, quality and performance following application of the specified sterilization process.

9 Validation

9.1 General

The purpose of validation is to demonstrate that the sterilization process established in process definition (see Clause 8) can be delivered effectively and reproducibly to the sterilization load. Validation consists of a number of identified stages, as follows:

— installation qualification (IQ);

— operational qualification (OQ);

— performance qualification (PQ).

IQ is undertaken to demonstrate that the sterilization equipment and any ancillary items have been supplied and installed in accordance with their specification (if appropriate). OQ is carried out either with unloaded equipment or using appropriate test material to demonstrate the capability of the equipment to deliver the sterilization process that has been defined.

PQ is the stage of validation that uses product (or surrogate product) to demonstrate that equipment consistently operates in accordance with predetermined criteria and the process produces a product that is sterile and meets the specified requirements.

NOTE If the storage solution is used as the liquid chemical sterilizing agent, the adequacy of the sterile barrier system as the container in which the sterilization process is delivered is considered during IQ, OQ and PQ in order to demonstrate suitability and reproducibility.

9.2 Installation qualification

9.2.1 Equipment

9.2.1.1 Equipment to be used in the sterilizing process, including ancillary items, shall be specified.

9.2.1.2 The operating procedures for the equipment shall be specified. These operating procedures shall include, but are not limited to:

a) step-by-step operating instructions;

b) fault conditions, the manner in which they are indicated and actions to be taken;

c) instructions for maintenance and calibration; and

d) details of contacts for technical support.

9.2.2 Installation

9.2.2.1 The location in which the equipment is to be installed, including any services required, shall be specified. Any special precautions and provisions shall be identified (e.g. safety equipment).

9.2.2.2 Instructions for installation shall be specified and shall include instructions pertinent to the health and safety of personnel.

9.2.2.3 If applicable, conditions for the safe storage of the liquid chemical sterilizing agent, to ensure that its quality and composition remain within specification, shall be documented.

9.2.2.4 Prior to OQ, the calibration status of all instrumentation (including any test instruments) used for monitoring, controlling, indicating or recording shall be confirmed.

9.3 Operational qualification

9.3.1 It shall be demonstrated that the equipment and any ancillary items, as installed, operate as intended.

9.3.2 OQ shall demonstrate that the installed equipment is capable of delivering the documented process (see 8.1) within specified tolerances.

9.4 Performance qualification

9.4.1 General

Physical performance qualification (PPQ) and microbiological performance qualification (MPQ) shall be conducted, and shall provide documented evidence that the equipment, as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and yields a product meeting its specification.

9.4.2 Microbiological performance qualification (MPQ)

9.4.2.1 General

9.4.2.1.1 MPQ shall demonstrate that the sterilization process delivers the specified sterility assurance level (SAL). This may be achieved using a procedure in accordance with 9.4.2.2 or 9.4.2.3. MPQ shall be performed in the production equipment, except as noted below. MPQ shall be performed using the combination of process parameters (e.g. liquid chemical sterilizing agent concentration, temperature, pH) that has been identified as producing the least lethality, given the specified tolerances. The rationale for selection of these process parameters shall be documented.

9.4.2.1.2 MPQ shall confirm the effectiveness of the defined process for the product/load combination in the production equipment.

9.4.2.1.3 If the process definition was not conducted in the production equipment using the product loaded as for production, the MPQ shall include at least three fractional or half cycles in the production equipment that confirm the data from the process definition. See Annex B for further information.

9.4.2.1.4 Product used in MPQ shall meet the following requirements:

a) The product (or surrogate product) shall be inoculated in the worst-case location with microorganism(s) demonstrated to be the most resistant to the process as identified in the process definition. If appropriate, microorganisms can be induced to grow on product.

b) For a product exposed to the liquid chemical sterilizing agent in an individual container, the worst-case sterilizing conditions for placement of the individual container(s) within the production equipment shall be represented.

c) For a product sterilized in bulk, it can be impracticable to perform PQ in the production equipment, as indicator organisms could be introduced into the manufacturing environment. In this case, a bulk container made of similar material, with a similar ratio of volume of liquid chemical sterilizing agent to the product, may be used. If large numbers of product units are routinely processed together, a smaller number of items may be used provided the volume of liquid chemical sterilizing agent per item is the same.

9.4.2.1.5 Microorganisms that have been inoculated onto a product, rather than induced to grow, could potentially be washed off during exposure to the liquid chemical sterilizing agent. Provision shall be made to evaluate the extent of microorganism wash-off for the MPQ.

9.4.2.2 Combined reference organism/bioburden approach

The inoculated product shall be exposed to a series of incremental exposures designed to deliver less lethality than the holding time used routinely. Rate of inactivation shall be calculated and from knowledge of the bioburden, it shall be verified that treatment required to achieve specified requirements for sterility is delivered in the time predicted in process definition. After time graded exposure(s), the lethality of the process may be determined using:

a) direct enumeration (inactivation curve);

b) the fraction negative method;

c) a combination of a) and b).

NOTE A detailed description of the procedure is given in B.1.

9.4.2.3 Overkill approach

9.4.2.3.1 Product inoculated with 10^6 reference microorganisms is exposed to either half the holding time [see 9.4.2.3.2], or a fraction of the holding time [see 9.4.2.3.3] predicted in the process definition.

9.4.2.3.2 For the half-cycle approach, three consecutive half-exposure cycles shall be performed.

No microorganisms shall be isolated after exposure to each of the half cycles. This is a simple way to demonstrate a greater than 6-$\log_{10}$ reduction in half the sterilization time.

As the reaction kinetics can be non-linear, some microorganisms could be isolated after exposure to the half cycles. For sterilization processes that do not demonstrate linear inactivation kinetics the exact nature of the inactivation kinetics shall be established in order to derive a relationship to use for the extrapolation. A validated enumeration shall be performed to demonstrate a 6-$\log_{10}$ reduction in organisms. Statistical treatment should be applied if the inactivation kinetics are not linear.

NOTE Additional guidance is provided in B.2.

9.4.2.3.3 For the cycle calculation 12 *D* approach, three consecutive fractional cycles shall be performed. A 12 *D* process shall be confirmed by calculating the log reduction of the full process from the extrapolation of the log reduction in the fractional cycle, based on the *D* values determined from the inactivation curves.

NOTE A detailed description of the procedure is given in B.1 and B.2.

9.4.3 Physical performance qualification

9.4.3.1 Conformity with process parameters for temperature established during process definition and reproducibility of temperature in the chamber and within individual product containers shall be determined.

9.4.3.2 The pH and concentration of the liquid chemical sterilizing agent(s) before and after the process shall be determined.

9.4.3.3 Loading pattern of individual packages to be sterilized shall be demonstrated to be suitable.

9.4.3.4 If bulk sterilization processes are used, then the temperature of the liquid chemical sterilizing agent shall be determined throughout the vessel and inside the product tissue in three consecutive process runs.

9.4.3.5 PQ shall include a series of at least three successful exposures of the product to the sterilization process, within specified tolerances, in order to demonstrate the reproducibility of the process. Results from PQ outside specified tolerances shall be reviewed and corrective actions determined and instituted before initiating a new series of exposures.

The series of three successful exposures shall be performed consecutively, unless findings outside specified tolerances can be attributed to factors not relevant to the effectiveness of the process being validated. Such findings shall be documented as unrelated to the performance of the sterilization process.

9.4.4 Aseptic processing qualification

If any aseptic manipulations (e.g. aseptic transfer of the medical device, aseptic transfer of solutions to/from the medical device final container), are conducted following completion of the sterilization process:

a) processes used for the sterilization of components for manufacture (e.g. finished product containers, equipment used in aseptic transfer operations, storage solutions) shall be validated and routinely controlled in accordance with the applicable International Standard (see Bibliography);

b) procedures after exposure to the liquid chemical sterilizing agent shall be validated in accordance with the applicable part(s) of ISO 13408.

9.5 Review and approval of validation

9.5.1 The purpose of this activity is to conduct and document a review of the validation data to confirm the acceptability of the sterilization process and to approve the process specification. When aseptic transfer is used as part of the manufacturing process, the validation data for this step are included.

9.5.2 Information gathered or produced during product definition, process definition, IQ, OQ and PQ shall be recorded and reviewed for acceptability. The results of this review shall be recorded.

9.5.3 A validation report shall be prepared. The report shall contain or reference a complete process specification, including the process parameters and their tolerances. The rationale for any deviation or modification to the process plan shall be justified and documented. The process specification shall detail the following:

a) the frequency and methods of determination of bioburden in accordance with ISO 11737-1, together with action levels;

b) the specification for the environment in which the liquid chemical sterilizing agent and containers are prepared and aseptic transfers are undertaken;

c) the training and competence criteria for approval of personnel to be authorized to undertake aseptic transfers;

d) the method of ensuring absence of viable microorganisms from the liquid chemical sterilizing agent solution(s);

e) the formulation of the liquid chemical sterilizing agent, including the specification of its constituents;

f) the pH of the liquid chemical sterilizing agent before and after the process;

g) the concentration of the liquid chemical sterilizing agent(s) before and after the process;

h) the specification of the production equipment in which products come into contact with the liquid chemical sterilizing agent, including materials of construction, size and details of pre-treatment, if applicable, to be applied to ensure the absence of viable microorganisms;

i) the number of products as characterized in the product definition to be sterilized per unit volume of liquid chemical sterilizing agent;

j) the holding time;

k) the temperature to be used for sterilization;

l) any other process variable(s) monitored during process definition;

m) the method of sterilizing any storage solution in which product is presented after sterilization.

9.5.4 In cases where a validation for a specific device is also judged valid for other devices, the justification for this shall be documented.

10 Routine monitoring and control

10.1 The purpose of routine monitoring and control is to demonstrate that the validated and specified sterilization process has been delivered to the product.

10.2 At defined intervals, the bioburden immediately preceding sterilization shall be determined as described in ISO 11737-1. If a microorganism that has not been studied in the microbicidal effectiveness studies (see 5.3) or the process definition (see 8.2) is isolated during routine determination of the pre-sterilization bioburden, the resistance characteristics of the isolate shall be assessed. If a new isolate could pose a greater challenge to the sterilization process, then the exercise in 5.3.2 shall be performed with this isolate.

10.3 Data shall be recorded and retained for each batch of sterilized product to demonstrate that the sterilization process specification has been met. These data shall include at least the following:

a) the process variables during the sterilization of final container(s);

b) the process variables monitored during the sterilization of the storage solution, if applicable;

c) the chemical concentration of the liquid chemical sterilizing agent(s) before and after the process;

d) the pH of the liquid chemical sterilizing agent before and after the process;

e) the process variables monitored during preparation of the liquid chemical sterilizing agent;

f) the results of integrity tests on any filters used to sterilize solutions, if applicable;

g) the sterilization holding time;

h) the temperature during the holding time;

i) the results of environmental monitoring for any aseptic transfer process, if applicable, in compliance with ISO 13408-1;

j) the records relating to preparing storage solutions and liquid chemical sterilizing agent solutions, controlling the sterilization process and any aseptic transfer process, if appropriate;

k) the serial numbers or other unique identifiers of product or product groups processed.

10.4 If parametric release is to be used (for terminally-sterilized product only), the following data shall also be recorded in addition to the requirements of 10.3:

a) the temperature during the holding time in two positions, the worst cases having been demonstrated in the validation studies (two independent sensors);

b) a confirmation that validated loading pattern for product was used;

c) a confirmation of the working of the circulation system, if used;

d) the results of the bioburden determination and the determination of resistance characteristics, if applicable, for each batch of tissue processed.

10.5 For conventional release of each batch of terminally-sterilized product, at least the following shall be examined for the presence of viable microorganisms:

a) the final storage solution; and

b) at least one of the following:

1) finished medical device;

2) product that has been rejected but subjected to the complete manufacturing process;

3) isolated piece of animal tissue or other component, or both, justified as being representative of the medical device, which have been subjected to the complete manufacturing process.

10.6 For each batch of product that is aseptically packaged after sterilization, the following shall be examined for the presence of viable microorganisms using a pharmacopoeial test for sterility:

a) the final storage solution; and

b) at least:

1) a finished product;

2) a product that has been rejected but subjected to the complete manufacturing process;

3) isolated piece of animal tissue or other component, or both, justified as being representative of the medical device, which have been subjected to the complete manufacturing process.

11 Product release from sterilization

11.1 One or more procedures for product release from sterilization shall be specified. The procedure(s) shall define the criteria for conformance.

a) For parametric release, the procedure shall include conformance to the process specification(s), as described in 10.3, and additional requirements as specified in 10.4. Parametric release of the product is only acceptable when a terminal sterilization process is used.

b) For conventional release of a terminally-sterilized product, the procedure shall include conformance to the process specification(s) as described in 10.3 and following incubation there shall be no growth in the microbiological testing described in 10.5.

c) For conventional release of a product that is sterilized in individual containers or sterilized in bulk, followed by an aseptic transfer of the product or solution, the procedure shall include conformance to the process specification(s) as described in 10.3 and, if applicable, 10.4, and following incubation there shall be no growth in the microbiological testing described in 10.6.

11.2 A given sterilization process shall be considered as non-conforming and the processed product handled as non-conforming product if:

a) a process variable is outside documented tolerances; or

b) any microbiological test shows growth following incubation (see 10.5. and 10.6).

12 Maintaining process effectiveness

12.1 General

The continued effectiveness of the system for ensuring the condition of the product presented for sterilization shall be demonstrated. This includes routine monitoring of product bioburden or monitoring the effectiveness of bioburden reduction steps, or both.

12.2 Maintenance of equipment

12.2.1 Preventative maintenance shall be planned and performed in accordance with documented procedures. The procedure for each planned maintenance task and the frequency at which it is to be carried out shall be specified and documented. Equipment shall not be used to process medical devices unless all specified maintenance tasks have been satisfactorily completed and recorded.

12.2.2 Records of maintenance shall be retained. The maintenance scheme, maintenance procedures and maintenance records shall be reviewed periodically by designated personnel.

12.3 Requalification

12.3.1 The validation and any subsequent requalification data shall be reviewed at least annually and a rationale shall be prepared and documented as to whether or not requalification is required and its extent.

12.3.2 A requalification exercise shall be undertaken unless sufficient data have been generated to demonstrate the continued appropriateness of the sterilization process. Procedures for the review of validation and requalification data shall be documented and records of requalification shall be retained. Requalification of a sterilization process carried out with specified equipment shall be performed at defined intervals against specified acceptance criteria and in accordance with documented procedures.

12.3.3 A requalification report shall be documented. The report shall be signed by the persons designated by the same functions/organizations that prepared, reviewed and accepted the original validation report.

12.3.4 Requalification for processes in parametric release shall be done at least annually and shall include MPQ and PPQ.

12.3.5 Frequency of requalification of aseptic transfer procedures after exposure to the liquid chemical sterilizing agent shall be in accordance with the applicable part of the ISO 13408 series.

12.4 Assessment of change

12.4.1 Any change in the sterilization equipment that could affect delivery of the sterilization process shall be assessed. If the sterilization process is judged to be affected, then a repeat of part or all of IQ, OQ or PQ shall be carried out. The outcome of this assessment, including the rationale for decisions reached, shall be recorded.

12.4.2 Any change in the product, its package or the presentation of the product for sterilization shall be assessed for the effect on the continued appropriateness of the sterilization process. Those parts of the process definition that have to be repeated shall be determined based on the nature of the change. The outcome of the assessment, including the rationale for the decisions reached, shall be recorded.

Annex A
(informative)

Guidance for the application of this document

A.1 Scope

The guidance given in this annex is not intended as a checklist for assessing compliance with this document.

NOTE For ease of reference, the numbering of clauses in this annex corresponds to that in the normative part of this document

This guidance provides explanations and methods suitable for achieving compliance with the specified requirements. This guidance is provided to assist in obtaining uniform understanding and implementation of this document. Methods other than those given in the guidance may be used. However, these methods should be demonstrated as being effective in achieving compliance with the requirements of this document.

The guidance is not intended to be exhaustive but is offered in order to highlight important aspects to which attention should be given. It provides examples of how to meet the requirements, recognizing that other methods that achieve the same ends are equally acceptable. It also gives general advice on how to meet the requirements and draws attention to aspects of the requirements that could not be readily apparent to those unfamiliar with the sterilization of medical devices.

A.2 Normative references

The requirements given in documents that are included as normative references are requirements of this document only to the extent that they are cited in normative parts of this document: the citation can be to a whole standard or limited to specific clauses.

A.3 Terms and definitions

No guidance provided.

A.4 General

A.4.1 Documentation

A.4.1.1 Requirements for the control of documents and records are specified in ISO 13485. In ISO 13485, the requirements for documentation relate to the generation and control of documentation (including specifications and procedures) and records. Procedures required to be specified by this document are established, documented, implemented and maintained.

Procedures are specified for characterization of a liquid chemical sterilizing agent, development, validation and routine control of a sterilization process and product release from sterilization.

Documents, including records, required by this document are reviewed and approved by designated personnel prior to issue and following change.

Documents are controlled to ensure that relevant versions of applicable documents are available for use.

Records are maintained to provide evidence of conformity to the requirements of this document. The controls needed for the identification, storage, security and integrity, retrieval, retention and disposition of records are specified.

The period for which documents, including records, are to be retained is specified.

The responsibility and authority for implementing and meeting the requirements described in this document are specified. Responsibility is assigned to competent personnel the basis of appropriate education, training, skills and experience. Requirements for responsibility and authority, and human resources are specified in ISO 13485. Requirements are specified for management responsibility related to management commitment, customer focus, quality policy, planning, responsibility, authority, communication and management review. The level of qualification, training and experience required by personnel will depend upon the activities being performed. General guidance on training as part of the overall quality management system is given in ISO 9004. Particular qualifications and training are appropriate for personnel with the responsibilities for:

a) microbiological testing;

b) chemical analysis and formulation;

c) installation of equipment;

d) equipment maintenance;

e) PPQ and MPQ;

f) routine sterilizer operation;

g) calibration;

h) process design;

i) equipment specification.

If the requirements of this document are undertaken by organizations with separate quality management systems, the responsibilities and authority of each party are specified in a written agreement.

Procedures for purchasing are specified to ensure that purchased product conforms to specified purchasing information.

Procedures for identification and traceability of product are specified. These procedures ensure the identification of the status of product throughout product realization. Identification of product status is maintained throughout production and storage to ensure that only product that has passed the required inspections and tests is dispatched.

Procedures for control of product designated as non-conforming and for correction, corrective action and preventive action are specified. These procedures ensure that product which does not conform to requirements is identified and controlled to prevent its unintended use or delivery. Records of the nature of the nonconformities and any subsequent action taken, including the evaluation, any investigation and the rationale for decisions are maintained.

Action is taken to eliminate the cause of nonconformities in order to prevent recurrence and of potential nonconformities to prevent occurrence. Any necessary corrective actions are taken without undue delay. Corrective actions and preventive actions are proportionate to the effects of the nonconformities encountered.

A.4.1.2 A calibration system complying with ISO 13485:2016, 7.6 or ISO 10012 is specified for the calibration of all equipment, including instrumentation for test purposes, used in meeting the requirements of this document.

A.5 Sterilizing agent characterization

A.5.1 The stages of liquid chemical sterilizing agent characterization through microbiological process definition include isolate collection, characterization, challenge test screening for resistance, identification of most resistant microorganism(s) representative of the bioburden for the sterilization process, construction of inactivation curves, and assessment of inactivation of the identified reference microorganism(s) on tissue carriers (see 5.3 and 8.2).

As it is often impractical to carry out inactivation studies on all isolates obtained from the product, a screening test should be employed. By exposing samples of tissue to conditions less severe than those used for processing, the more resistant isolates can be quickly segregated and used in the inactivation studies.

Due to the unique application of liquid chemicals for use in sterilization processes, it is necessary to be vigilant in detecting, screening and testing microorganisms which are found to be present and which could pose a significant resistance to the sterilization process. A risk exists that new or altered microorganisms could be introduced during the manufacturing process and could possess a resistance to the sterilization process that is greater than the original test and validation microorganism(s). Therefore, an ongoing procedure for screening and evaluating the resistance of microorganisms encountered in the manufacturing process and environment should be established (microbiological isolate screening procedure). The microbiological screening process should be conducted to ensure that new or modified microorganisms are detected and evaluated in a timely manner.

A.5.2 The microbiological isolate screening procedure should incorporate the following three phases:

a) *Microbiological isolate collection*: microbiological isolates of interest should be collected from the manufacturing process and environment in which the medical device is produced. Collection should concentrate initially on microorganisms that are known to exist on the product prior to sterilization and that could be capable of surviving exposure to the solutions used in the manufacturing process (process or bioburden isolates). In addition to the product bioburden, isolates should be collected from the manufacturing environment in which the product is produced if it is of the type that could be capable of survival in the process chemicals. This environment can include, but is not limited to, process solutions, work surfaces, water purification systems, raw materials and personnel. See Table A.1 for examples of additional classes of microorganisms to consider.

b) *Microbiological isolate characterization:* microbiological isolates collected for evaluation should be characterized or identified, or both, for future reference. Characterization should include, at a minimum, colony morphology, cellular morphology, Gram reaction and growth rate description. When possible, identification of the species or subspecies is preferred.

c) *Challenge testing (screening):* challenge testing of the microbiological isolates should be conducted as indicated in 5.3.2. Microbiological isolates which demonstrate a significant resistance to the sterilization process during initial challenge testing should be evaluated fully for lethal rate and compared to the microorganism(s) used for initial process definition and validation studies. The relative resistance of the challenge microbiological isolates should be evaluated with regard to the overall sterilization process.

Approaches to initial challenge testing include:

— exposing tissue samples or a suspension of isolated microorganisms (10^5 cells) to the liquid chemical sterilizing agent at minimum conditions of the process specification for a fraction of the time to inactivate a similar population of the most resistant microorganism used in PQ. If, following this exposure, survivors are detected, this indicates that the resistance of the isolate could be similar to that of the most resistant microorganism used in PQ. The isolate should be subjected to full characterization and detailed investigation of its inactivation kinetics;

— exposing a suspension of isolated microorganisms containing at least 10^5 cells to the liquid chemical sterilizing agent for a time equal to one to two times the *D* value of the reference microorganism. Detection of survivors would indicate that the *D* value of the isolate is at least 20 % to 40 %

(respectively) of the *D* value of the reference microorganism. Isolates that are resistant to this limited exposure should be subjected to inactivation studies to determine the *D* value in suspension. If the *D* value determined under these conditions is greater than 50 % of the *D* value of the reference microorganism, the isolate(s) should be subjected to characterization and determination of inactivation kinetics in the presence of product (or representative carriers) as described in 8.2.4.

A.5.3 A reference microorganism(s) is selected for qualification which is at least representative of the resistance of the bioburden prior to the final sterilization process. The microorganisms selected and the rationale for their choice should be recorded.

The resistance of the selected reference microorganism should be at least as great as *Bacillus atrophaeus.* The reference microorganism should be designated by a recognized culture collection reference or other identifier that allows the source to be traced.

Examples of microorganisms which have been employed for characterization are given in Table A.1. The microorganisms listed have previously demonstrated significant resistance to a liquid chemical sterilizing agent with animal tissues. The table is provided as reference and guidance only and is not intended to be a list of microorganisms required to be evaluated.

See also Annex C for the activities for, and the interrelationships of, microbicidal effectiveness, process definition and microbiological performance qualification.

A.5.4 The selection of species of reference microorganisms to be used in the validation of a liquid chemical sterilization process for material of animal origin should take into account test screening for isolates under 5.3.2 and those representing potential bioburden which can possess some resistance to the process chemicals, including the following:

1) microbiological species which could be present as a result of the source of the animal tissue;

2) microbiological species isolated during the determination of bioburden on product;

3) microbiological species isolated from the process or environment in which the animal tissue is harvested and the manufacturing environment in which the final medical device is produced;

4) microbiological species which have a demonstrated high resistance to the liquid chemical sterilizing agent or which can be expected to have an increased resistance and which are of potential impact to the process sterilization stage;

5) a range of microbiological species.

Table A.1 — Examples of microorganisms which have been used for assessment of the activity of specific liquid chemical sterilizing agents

Species	Culture collection number		
	ATCC[a]	NCTC[b]	NCIMB[c]
Spores of:			
Clostridium sporogenes	3584	—	10696
Bacillus subtilis	6051	3610	3610
Bacillus atrophaeus	9372	—	—
Bacillus pumilus	27142	10327	10692
Chaetomium globosum	6205	—	—
Microascus cinereus	16594	—	—
Vegetative cells of:			
Mycobacterium chelonae	35752	946	1474
Methylobacterium extorquens	43645	—	9399
Trichosporon aquatile	22310	—	—

NOTE This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. As an alternative, similar or equivalent culture held by culture collections registered under the Budapest Convention can be used.

[a] American Type Culture Collection.

[b] National Collection of Type Cultures.

[c] National Collection of Industrial and Marine Bacteria.

A.6 Process and equipment characterization

No guidance provided.

A.7 Product definition

A.7.1 The development of a sterilization process for a particular medical device needs to establish a process that is both effective and compatible with the medical device. Therefore, initial investigations into product compatibility, together with experimentation to identify or optimize the sterilization process, or both, should be undertaken while product is in the design phase.

Particular attention should be given to the specific aspects related to the fact that the product is manufactured from animal tissues.

A.7.2 During a liquid chemical sterilization process, the product can be subjected to environmental stresses. The product could also react with the liquid chemical sterilizing agents. The design of the product should ensure that functionality and safety are not compromised by exposure to the anticipated range of sterilization conditions.

If the medical device is sterilized in the final container, the validation data for the sterilization of the container should be included, demonstrating that the sterilization process(es) used to sterilize the container have been validated and are routinely controlled in accordance with the appropriate International Standard.

For some products, contamination by organic material can be a contributory factor in limiting process efficacy. The investigations should include evaluations of solutions containing appropriate organic material (e.g. serum, albumin, etc.) or a sterile macerated suspension of tissue. The type and concentration of organic material should be documented. For products where drying in the presence of organic material can occur, inoculated carriers comprising microorganisms dried in suitable organic material should also be used. Such inoculated carriers should include preparations of microorganisms

resistant to drying, such as *Clostridium sporogenes* spores, *Bacillus atrophaeus* spores, *Enterococcus faecalis, Mycobacterium chelonae* and *Candida albicans* (see A.8).

Extraneous organic and inorganic matter can interfere with the action of liquid chemical sterilizing agents. Processing and inspection procedures should be in place to ensure removal of visible contaminants, such as blood and extraneous tissue. 7.2 is not intended to establish a requirement for quantitative analysis for organic and inorganic contaminants.

A.7.3 No guidance provided.

A.7.4 This guidance is provided in addition to that described in ISO 11737-1 because of the particular difficulties in performing bioburden determinations of animal tissues.

The objective of bioburden determinations is threefold, as follows:

a) to establish the nature of contaminating microorganisms present;

b) to establish the number of microorganisms present;

c) to establish the extent of variation in contamination by comparing the numbers found on consecutive batches.

Bioburden determinations should be carried out on:

— starting materials of animal origin;

— materials after each significant processing stage;

— the product immediately prior to sterilization.

Any method for determining the bioburden will only indicate the presence of a limited proportion of the numbers and species of microorganisms present. Bioburden values should therefore be corrected for errors in the efficiency of the removal of microorganisms from the product and the effectiveness of the applied culture conditions in detecting those microorganisms that have been removed. Estimates of recovery efficiencies can vary and can be extremely low for animal tissues. As a conservative approach, the equation for calculation of holding time in 8.2.5 includes an additional safety factor of 100 to the estimate of the bioburden to compensate for these limitations.

Attention should be paid to the selection of appropriate media and incubation conditions for enumeration. See Table A.2 for examples of media, incubation conditions and corresponding microorganisms that can be used for growth promotion. In particular, the requirements for the isolation of microorganisms that can be associated with materials of animal origin should be considered.

Table A.2 — Summary of media, incubation conditions and microorganisms suitable for growth promotion

	Medium[a]	Possible incubation conditions[c]	Challenge organisms to demonstrate ability to support growth[d]
1	Tryptone soya broth and tryptone soya agar	Aerobic 30 °C–35 °C	*Bacillus atrophaeus* *Bacillus subtilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Brevundimonas diminuta* *Staphylococcus epidermidis*
2	Nutrient broth and nutrient agar	Aerobic 30 °C–35 °C Aerobic 20 °C–25 °C	*Bacillus atrophaeus* *Bacillus subtilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Brevundimonas diminuta* *Saccharomyces cerevisiae*
3	Lowenstein-Jensen	Aerobic 30 °C–35 °C	*Mycobacterium phlei (3 weeks)*
4	Blood agar	Aerobic 30 °C–35 °C Anaerobic 30 °C–35 °C	*Bacillus atrophaeus* *Bacillus subtilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Brevundimonas diminuta* *Staphylococcus epidermidis* *Enterococcus faecalis* *Clostridium sporogenes*
5	Potato dextrose agar	Aerobic 20 °C–25 °C	*Saccharomyces cerevisiae* *Trichophyton rubrum*
6	Robertson's cooked meat broth[b]	Aerobic 30 °C–35 °C	*Clostridium sporogenes*

NOTE 1 Organisms recommended in Pharmacopoeias can also be used for growth promotion tests.

NOTE 2 Recovery of one or more colony forming units of the inoculate microorganism (or visible growth in liquid medium) would be regarded as satisfactory evidence of the medium's ability to support growth of the test organism. In testing liquid media, small volumes should be employed (e.g. 10 ml) if dilution inhibition is to be avoided.

Footnotes:

[a] Media should not incorporate colour change indicator dyes (which are frequently inhibitory to microbiological growth) except in special circumstances, e.g. L-J medium. The detection of growth in liquid media should be made visually either by turbidity or by special density/microscopy and, where necessary, confirmed by subculture on appropriate solid media.

[b] Alternative media with reducing properties suitable for detection of anaerobic growth may be substituted.

[c] All incubation for growth promotion should be for a minimum period of two weeks except L-J medium which should be for a minimum period of three weeks. Temperature should be controlled within tolerances selected from within the range given. Injured organisms can require a longer incubation period.

[d] Each batch of a medium should be tested to demonstrate that the medium is capable of recovering 10–100 microorganisms per inoculum (a batch should be regarded as a single preparation from one batch of raw materials prepared at one time). Testing should be done with reference microorganisms from recognized culture collection.

If an inoculum of 10 microorganisms is used, this will rarely lead to the recovery of 10 colony forming units (cfu) on solid media, because:

1) there will be a difference between the total and viable count; and

2) the precise number of organisms in the inoculum will vary by approximately a mean of 10.

A.8 Process definition

A.8.1 Process definition is undertaken to define the process parameters for a sterilization process, which will achieve the specified requirements for sterility for a specified product without adversely affecting product functionality. The selection of the sterilization process to be used for medical devices should include consideration of all factors which influence the efficacy of the process. The following could be taken into account:

a) the availability of sterilization equipment;

b) the range of conditions that can be achieved with the sterilizing equipment available;

c) the sterilization processes already in use for other products;

d) the requirements for levels of residual chemicals or their reaction products, or both;

e) the results of process definition experiments.

A process definition exercise consists of a number of elements, during which appropriateness of the inoculated carrier to be used for PQ and routine monitoring should be confirmed. As a result of the process definition activities, a sterilization process can be specified. The appropriateness of this sterilization process with the product load is confirmed in the PQ studies.

See also Annex C for the activities for, and the interrelationships of, microbicidal effectiveness, process definition and microbiological performance qualification.

A.8.2 Process definition is required to provide an inactivation curve for the reference microorganism(s) with a minimum of five points covering at least a thousandfold reduction in numbers (Clause 8). See B.1.

The rationale for selection of these process parameters should be documented and should take into account the combination of process parameters shown to be present throughout the sterilization process. Liquid chemical sterilization is a dynamic process in which the critical process parameters change from the start to the end of exposure. Chemical concentration and pH changes can occur due to dilution or reaction with the product. Temperature can increase (or decrease) as a result of heat transfer. Hence, the least lethal temperature could be observed at the start of the process, and the least lethal chemical concentration could be observed at the end.

A.8.3 The simplest and most direct approach to process definition would be to select the values for each process parameter, giving the lowest lethality expected at any point in the exposure, and to conduct the inactivation kinetics studies using that combination of parameters.

A.8.4 Process definition of liquid chemical sterilization processes should be conducted so that the possible interactions of the test microorganism(s) with the medical device can be evaluated as described in Clause 8.

Conducting such evaluations requires the investigator to perform four essential phases of investigation. These phases are:

a) determining components which represent the greatest challenge to the liquid chemical sterilizing agent (e.g. mated surfaces, porous matrix, crevices in the surface);

b) defining the method for inoculating the microorganism(s) on the medical device or selected component;

c) validating the method of recovery/detection of the test microorganism from the medical device or selected component;

d) determining the inactivation kinetics of the test organism(s) in the presence of the medical device or selected component.

Establishing reference microorganisms on tissue substrate/carriers: using the reference microorganism(s) selected that have demonstrated similar or greater resistance (as compared to bioburden) to the liquid chemical sterilizing agent in suspension tests, the test organism inactivation kinetics should be evaluated in the presence of the test component. If the location of the challenge is other than the most difficult-to-sterilize location, its relationship to the most difficult location should be established. The study design should include controlled ("worst-case") exposures of the inoculated or cultured test component in the liquid chemical sterilizing agent against time. Samples should be removed at predetermined time intervals to allow for estimation of the surviving population by the validated recovery method.

Studies into microbiological inactivation kinetics should be conducted on carrier materials that pose the greatest challenge to the sterilization process. Selection of the carrier should take into consideration the contact and/or interaction of the liquid chemical sterilizing agent with the carrier (e.g. hydrophobic, filamentous material can be a greater challenge than smooth, hydrophilic surfaces). The carrier should be representative of or equivalent to the actual tissue substrate in the medical device.

If the choice of carrier is not readily apparent, screening tests to identify the most challenging carrier should be performed.

The method of inoculating viable microorganisms on the carrier prior to exposure to the liquid chemical sterilizing agent is paramount in creating a simulation that will assess the sterilization process effectively and in a manner that estimates challenges to the sterilization processes as they occur in practice. Two methods can be used to establish a viable microorganism on the test component: direct inoculation or cultivation in simulated manufacturing conditions.

Direct inoculation utilizes a viable spore or cell suspension that is applied to the carrier or medical device component(s) immediately prior to the liquid chemical sterilizing agent exposure. This approach should be used for reference microorganism(s) representing the bioburden which would not survive to co-exist with the material during storage in the process solutions.

Consideration should be given to the time that the inoculum is allowed to penetrate and adhere to the carrier prior to liquid chemical sterilizing agent exposure. In addition, any bactericidal effect of the carrier should be taken into account.

When reference microorganisms are used for direct inoculation onto sites that can confer resistance, provision should be made to account for any wash-off of the test organism inoculum that can occur during the testing for development of the inactivation curve or MPN data. In order to account for reduction in inoculum, it is desirable to determine the proportion of the inoculum that can wash off the intended inoculum site.

Cultivation of microorganism(s) in simulated manufacturing conditions should be utilized for microorganisms that can co-exist with the material during storage in the manufacturing process solutions. This can permit significant proliferation; this method is preferred when possible over inoculation, when the selected microorganism is capable of growth in and/or on the product during normal manufacturing conditions. A culture of the carrier(s) with the test organism should be prepared. Under such conditions, the number of viable microorganisms present in and/or on the product needs to be at least 1 000 colony forming units and be uniform from one component to the next in a single culture system prior to liquid chemical sterilizing agent exposure. Data to demonstrate appropriate minimum population and uniformity should be collected immediately prior to initiating inactivation kinetics studies.

The method for recovering viable microorganisms from the carrier after exposure to the liquid chemical sterilizing agent should be specified and validated. The validation should demonstrate that the method chosen recovers the surviving organisms in a reproducible manner.

Test methods can be conducted by either direct enumeration to establish the number of survivors over time (inactivation curve) or by Most Probable Number (MPN) estimation (e.g. Stumbo, Murphy, Cochran or Spearman Karber methods); see ISO 11737-1 and Reference [21]. Inactivation curve construction is preferred, as this method provides sufficient data to allow the determination of the inactivation kinetics of the test microorganism over time in the presence of the medical device or selected component. MPN

methods could be necessary if the medical device or component does not allow consistent removal for enumeration of the surviving population over time (i.e. if the medical device or carrier cannot be macerated to allow estimation of the surviving microbiological population).

While direct enumeration and construction of an inactivation curve provide greater information about the inactivation kinetics of the test organism, they can also require more time and resources to perform. Direct enumeration procedures attempt to remove all viable test organisms after predetermined times of liquid chemical sterilizing agent exposure. Removal can be accomplished as for bioburden determination (see ISO 11737-1), for example by homogenizing the test component and preparing appropriate dilutions for survivor enumerations; however, this method is not applicable if there are materials in the product which are not readily homogenized.

See B.1 for additional guidance on performance of lethal rate studies.

A.8.5 As a conservative approach, the equation for calculation of holding time in 8.2.5 includes an additional safety factor of 100 to the estimate of the bioburden to compensate for these limitations.

A.8.6 Before commencing an MPQ, it is necessary to ensure that the results of qualification experiments are not adversely influenced by microbicidal or microbiostatic effects due to carry-over of the liquid chemical sterilizing agent into the recovery system. The effects of microbicidal or microbiostatic substances can be reduced by dilution, removed by filtration or inactivated by reaction with a neutralizing agent.

The choice of neutralization system will be influenced by the composition of the liquid chemical sterilizing agent, and the effectiveness of the chosen system should be demonstrated prior to the commencement of PQ.

A.9 Validation

Where the medical device is sterilized in the final container, the validation data which demonstrate that the processes used to sterilize the container have been validated and are routinely controlled in accordance with the appropriate International Standard should be included.

Specification of the holding time requires attainment of the specified load. Physical process qualification demonstrates the relationship between the period for which the temperature is maintained at the measurement point and the period for which the temperature is maintained at the worst-case location(s) in the load. Temperature in routine operation is commonly monitored at a convenient measurement point (i.e. location in the chamber, vessel, or load), but not throughout the load.

See also Annex C for the activities for, and the interrelationships of, microbicidal effectiveness, process definition and microbiological performance qualification.

A.10 Routine monitoring and control

A.10.1 No guidance provided.

A.10.2 See A.7.4 for general guidance on bioburden determination during process validation.

Ongoing microbiological monitoring: microbiological isolation and challenge testing should be conducted on a routine basis. The goal of such testing is to detect possible changes to the microorganism(s) that can be present during the sterilization process. Since validation procedures are established to evaluate the sterilization process against a given range of microorganisms, ongoing testing should be performed to provide evidence that the microorganisms presented to the sterilization process are not or have not become more resistant than those used during original validation studies.

A.10.3 A sterilization process using liquid chemical sterilizing agents usually involves a number of phases, such as:

a) the preparation of the liquid chemical sterilizing agent;

b) the exposure of the product to the liquid chemical sterilizing agent at a controlled temperature for a specified time;

c) the sterilization of the primary package.

If it is not a terminal sterilization process, there are two additional phases:

— the preparation and sterilization of any storage solution in which product is to be presented;

— the aseptic transfer of the product from the liquid chemical sterilizing agent into its primary pack or aseptic removal of the liquid chemical sterilizing agent from the primary pack and replacement with storage solution.

The preparation of the liquid chemical sterilizing agent requires careful control. Records of the constituents, such as batch numbers and quantities, should be retained and the final concentration of the active ingredient(s) should be confirmed by assay. Frequently, liquid chemical sterilizing agents are filtered prior to use in order to remove any microorganisms and other impurities carried over from the components of the liquid chemical sterilizing agent. Filters used for such processes should be tested for their integrity after use.

The sterilization process is required to be carried out in exposure vessels of defined specification under temperature-controlled conditions.

In order to assess the routine acceptability of a process, the composition of the liquid chemical sterilizing agent post-sterilization should be checked after the product has been removed. The chemical composition of the liquid chemical sterilizing agent should be assayed following completion of the process to confirm that the composition remains within specification. A microbiological check, for example, could be exposure of an inoculated carrier to the liquid chemical sterilizing agent to demonstrate continued microbicidal efficacy. The liquid chemical sterilizing agent may be collected either as a random sample from the product batch, or from items designed to be representative of the batch, and which are sterilized along with the batch (e.g. scrapped surrogate product).

Following exposure, the product can be transferred aseptically to its final container or the liquid chemical sterilizing agent could be aseptically removed and replaced with a storage solution. During this transfer, the environment in the vicinity should be monitored microbiologically.

A list of personnel who have been qualified to undertake aseptic transfers should be approved, established and maintained. This list should be kept under constant review and personnel should be requalified at defined intervals. The qualification and requalification typically takes the form of media transfers and parallels the approach of "broth fills" ("media fills") used to qualify filtration sterilization and aseptic processes.

If product is to be presented in a storage solution, the storage solution should be sterilized prior to use. If aseptic processing is used, ISO 13408-1 should be followed.

For further information on aseptic transfer, if applied, see ISO 13408-7.

A.10.4 Temperature of the sterilization load should be representative of the worst-case temperature of product.

A programme to monitor for the level and resistance of the bioburden of the animal tissue or its derivative should include monitoring sampling which is representative of processes for tissue sourcing, receiving and chemical processing. Resistance evaluation should be included during performance of bioburden studies and during culturing of the material after it has been exposed to chemical processing.