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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

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 14160 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 14160:1998), which has been technically revised.

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) may, prior to sterilization, have microorganisms on them, 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 may survive 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 International Standard 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 may 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 might 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 International Standard 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 may 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 International Standard 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 International Standard 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 International Standard is not applicable to material of human origin.

This International Standard does not describe methods for the validation of the inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents (see Note 2).

This International Standard 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 International Standard 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 International Standard 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 International Standard does not cover the level of residual sterilizing agent within medical devices (see Note 6).

This International Standard does not describe a quality management system for the control of all stages of manufacture (see Note 7).

NOTE 1 The prior application of risk management principles to medical devices utilizing animal tissues, as described in ISO 22442-1, is important.

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 International Standard 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 defined 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 referenced documents are indispensable for the application 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 10012, *Measure management systems — Requirements for measurement processes and measuring equipment*

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 medical devices — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

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

ISO 13485:2003, *Medical devices — Quality management systems — Requirements for regulatory purposes*

ISO 22442-2, *Medical devices utilizing animal tissues and their derivatives — Part 2: Controls on sourcing, collection and handling*

3 Terms and definitions

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

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

[ISO/TS 11139:2006, definition 2.1]

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

[ISO/TS 11139:2006, definition 2.2]

3.3
carrier
supporting material on or in which test microorganisms are deposited

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

[ISO/TS 11139:2006, definition 2.11]

3.5
exposure time
period for which the process parameters are maintained within their specified tolerances

[ISO/TS 11139:2006, definition 2.18]

3.6
inactivation
loss of the ability of microorganisms to grow and/or multiply

[ISO/TS 11139:2006, definition 2.21]

3.7
inoculated carrier
supporting material on or in which a defined number of viable test organisms have been deposited

3.8
installation qualification
IQ
process of obtaining and documenting evidence that equipment has been provided and installed in accordance with its specification

[ISO/TS 11139:2006, definition 2.22]

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

3.10
medical device
instrument, apparatus, implement, machine, appliance, implant, *in vitro* reagent or calibrator, software, material, or other related article, intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific purposes 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 for medical purposes by means of *in vitro* examination of specimens derived from the human body;

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

[ISO 13485:2003, definition 3.7]

NOTE This definition from ISO 13485:2003 was developed by the Global Harmonization Task Force (GHTF 2002).

3.11
operational qualification
OQ
process of obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures

[ISO/TS 11139:2006, definition 2.27]

3.12
parametric release
declaration that product is sterile, based on records demonstrating that the process parameters were delivered within specified tolerances

[ISO/TS 11139:2006, definition 2.29]

3.13
performance qualification
PQ
process of obtaining and documenting evidence that the equipment, as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and thereby yields product meeting its specification

[ISO/TS 11139:2006, definition 2.30]

3.14
product family
group or subgroup of product characterized by similar attributes such as mass, material, construction, shapes, lumens and packaging system, and which presents a similar challenge to the sterilization process

[ISO 17665-1:2006, definition 3.38]

3.15
requalification
repetition of part of validation for the purpose of confirming the continued acceptability of a specified process

[ISO/TS 11139:2006, definition 2.40]

3.16
specify
stipulate in detail within an approved document

[ISO/TS 11139:2006, definition 2.42]

3.17
sterile
free from viable microorganisms

[ISO/TS 11139:2006, definition 2.43]

3.18
sterility
state of being free from viable microorganisms

[ISO/TS 11139:2006, definition 2.45]

NOTE In practice, no such absolute statement regarding the absence of microorganisms can be proven (see 3.19).

3.19
sterilization
validated process used to render a product free from viable microorganisms

NOTE In a sterilization process, the nature of microbiological 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.

[ISO/TS 11139:2006, definition 2.47]

3.20
storage solution
liquid in which a medical device in its final form is presented for use

3.21
surrogate product
simulation of the item to be sterilized that presents an equal or greater challenge to the sterilization process

3.22
test for sterility
technical operation defined in an official pharmacopoeia performed on product following exposure to a sterilization process or following an aseptic manufacturing process

[ISO/TS 11139:2006, definition 2.53]

3.23
tissue
organization of cells, cells and extra-cellular constituents, or extra-cellular constituents

3.24
validation
documented procedure for obtaining, recording and interpreting the results required to establish that a process will consistently yield product complying with predetermined specifications

[ISO/TS 11139:2006, definition 2.55]

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

4 Quality management system elements

4.1 Documentation

4.1.1 Procedures for the development, validation, characterization and routine control of the sterilization process and for product release from sterilization shall be specified.

4.1.2 Documents and records required by this International Standard shall be reviewed and approved by designated personnel (see 4.2.1). Documents and records shall be controlled in accordance with the applicable clauses of ISO 13485.

4.2 Management responsibility

4.2.1 The responsibility and authority for implementing and meeting the requirements described in this International Standard shall be specified. Responsibility shall be assigned to competent personnel in accordance with the applicable clauses of ISO 13485.

4.2.2 If the requirements of this International Standard are undertaken by organizations with separate quality management systems, the responsibilities and authority of each party shall be specified.

4.3 Product realization

4.3.1 Procedures for purchasing shall be specified. These procedures shall comply with the applicable clauses of ISO 13485.

4.3.2 Procedures for identification and traceability of the product shall be specified. These procedures shall comply with the applicable clauses of ISO 13485.

4.3.3 Controls on the sourcing, collection and handling of animal tissues and their derivatives shall be performed in accordance with ISO 22442-2.

4.3.4 A system complying with the applicable clauses of ISO 13485 or ISO 10012 shall be specified for the calibration of all equipment, including instrumentation for test purposes, used in meeting the requirements of this International Standard.

4.4 Measurement, analysis and improvement — Control of non-conforming products

Procedures for control of products designated as non-conforming and for correction, corrective action and preventive action shall be specified. These procedures shall comply with the applicable clauses of ISO 13485.

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 sterilizing agent has on materials, and identify requirements for the safety of personnel and protection of the environment.

5.2 Sterilizing agent

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

a) the formulation of a sterilizing solution, including concentration of the active agent 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 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 sterilizing agent against a range of representative microorganisms,

NOTE Guidance on microorganism selection is included in A.6.2 and in Table A.2.

b) identify the process variables that affect the lethal action of the 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 sterilizing agent, and those that can influence its effectiveness [i.e. the 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 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 sterilizing agent.

5.4 Effects on materials

5.4.1 The effects of exposure to the sterilizing agent on the physical, chemical, or physical and 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 sterilizing agent.

5.4.2 If the product is to be exposed repeatedly to the 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 (see 4.1.2), together with the criteria against which the properties of materials were assessed before and after exposure to the sterilizing agent.

5.5 Safety and the environment

5.5.1 Either a material safety data sheet (MSDS) or analogous safety information shall be specified for the sterilizing agent. This MSDS may be provided by a supplier for a chemical agent or be prepared as a prelude to experimental studies on the 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 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 (see 4.1.2).

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 be based upon knowledge of the combination of process parameters yielding the minimum effectiveness and shall 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 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 sterilizing agent (see 5.2) is provided, including any additives or precursors necessary for its delivery (see also 5.2.1),

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 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 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 resterilization 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 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 B.1), and hence establishment of the process parameters. An empirical mathematical relationship defining the microbiological 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.

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 exposure 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 sterilizing agent of an isolate from the bioburden or manufacturing environment may approach or exceed 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 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. sterilizing agent concentration, temperature and pH) that has been identified as producing the lowest lethality during the sterilizing agent characterization (see 5.3). 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 presterilization bioburden should be induced to grow on the tissue carriers, if possible (see B.1).

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 survivor 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 exposure time shall not be less than $D[6 + \log_{10}(100 + B)]$, where D is the D value of the most resistant microorganism identified above (see 8.2.4), and B is the value of the bioburden determined using ISO 11737-1. If the overkill approach is used in microbiological performance qualification (MPQ, see 9.4.2 and B.2), the sterilization exposure time defined 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 (see 8.2.2, 8.2.3 and 8.2.4) 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 exposure 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 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.

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 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 (see 4.3.4).

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 defined 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 shall demonstrate that the sterilization process delivers the specified sterility assurance level (SAL). This may be achieved using a procedure in accordance with 9.4.2.1 or 9.4.2.2. MPQ shall be performed in the production equipment, except as noted below. MPQ shall be performed using the combination of process parameters (e.g. 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.

MPQ shall confirm the effectiveness of the defined process (see 8.2.5) for the product/load combination in the production equipment.

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 8.2.1). See B.1 and B.2 for further information.

a) The product (or surrogate product) shall be inoculated in the worst-case location with organism(s) demonstrated to be the most resistant to the process as identified in the process definition (see 8.2.1). If appropriate, microorganisms can be induced to grow on product. See A.6.5.

b) For a product exposed to the 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 might not be possible 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 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 sterilizing agent/item is the same.

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

9.4.2.1 Combined reference organism/bioburden approach

The inoculated product shall be exposed to a series of incremental exposures designed to deliver less lethality than the exposure 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 (8.2.5). After time graded exposure(s), the lethality of the process can be determined using:

a) direct enumeration (survivor curve);

b) the fraction negative method;

c) a combination of a) and b).

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

9.4.2.2 Overkill approach

Product inoculated with 10^6 reference microorganisms is exposed to either half the exposure time [see item a) below], or a fraction of the exposure time [see item b) below] predicted in the process definition (see Clause 8).

a) *Half-cycle approach.* Three consecutive half-exposure cycles shall be performed.

   1) No organisms 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 (see 8.2.5).

2) As the reaction kinetics might not be linear, some organisms might 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 reaction kinetics are not linear.

Additional guidance is provided in B.2.

b) *Cycle calculation 12 D approach.* Three consecutive fractional cycles shall be performed. Confirm a 12 D process 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.

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 (see 8.2.1) 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 sterilizing agent temperature throughout the vessel and inside the product tissue shall be determined 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 defined tolerances, in order to demonstrate the reproducibility of the process. Results from PQ outside defined 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 defined 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 the medical device is subjected to an aseptic transfer 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 appropriate International Standard (see Bibliography);

b) transfer procedures after exposure to the liquid chemical sterilizing agent shall be validated in accordance with the appropriate part 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 limits;

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

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

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

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

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

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

h) the specification of the production equipment in which products come into contact with the sterilizing agent, including materials of construction, size and details of pretreatment, 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 sterilizing agent;

j) the exposure 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 presterilization bioburden, the resistance characteristics of the isolate shall be assessed. If a new isolate might 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 exposure time;

h) the temperature during the exposure 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 exposure 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 one item from the sterilization lot shall be examined for the presence of viable microorganisms and shall include:

a) the final storage solution; and

b) at least one of the following:

   1) a finished product;

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

   3) isolated pieces of animal tissue or other device components, 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 one of the following:

   1) a finished product;

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

3) isolated pieces of animal tissues or other components, 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.

11.1.1 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 a), b) and c). Note that parametric release of the product is only acceptable when a terminal sterilization process is used.

11.1.2 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 10.5.1.

11.1.3 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 following incubation there shall be no growth in the microbiological testing described in 10.5.2.

11.2 A given sterilization process shall be considered as non-conforming and non-conforming product shall be handled as specified in ISO 13485, if:

a) a process variable is outside documented tolerances; or

b) any microbiological test shows growth following incubation (see 10.5.1 and 10.5.2).

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 as specified in ISO 13485. 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 ISO 13408.

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 International Standard

A.1 General considerations

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

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 International Standard. 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 International Standard.

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 might not be readily apparent to those unfamiliar with the sterilization of medical devices.

The clauses and subclauses in this International Standard to which the guidance in this annex specifically applies are indicated by the number of the relevant subclause in square brackets.

A.2 Normative references [Clause 2]

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

A.3 Quality management system elements [Clause 4]

A.3.1 Documentation [4.1]

Requirements for the control of documents and records are specified in ISO 13485:2003, 4.2.3 and 4.2.4 respectively. In ISO 13485, the requirements for documentation relate to the generation and control of documentation (including specifications and procedures) and records.

A.3.2 Management responsibility [4.2]

Requirements for responsibility and authority are specified in ISO 13485:2003, 5.5, and requirements for human resources in ISO 13485:2003, 6.2. In ISO 13485, the 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.

A.4 Sterilizing agent characterization [Clause 5]

Guidance on microbicidal effectiveness screening studies is provided in A.6.2.

A.5 Product definition [Clause 7]

A.5.1 [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.5.2 [7.2] During a liquid chemical sterilization process, the product can be subjected to environmental stresses. The product could also react with the 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.6).

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. Subclause 7.2 is not intended to establish a requirement for quantitative analysis for organic and inorganic contaminants.

A.5.3 [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 may vary and can be extremely low for animal tissues. As a conservative approach, the equation for calculation of exposure 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. In particular, the requirements for the isolation of microorganisms that might be associated with materials of animal origin should be considered. See Table A.1.

Table A.1 — Summary of media, incubation conditions and organisms 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*

Table A.1 (*continued*)

	Medium[a]	Possible incubation conditions[c]	Challenge organisms to demonstrate ability to support growth[d]
5	Potato dextrose agar	Aerobic 20 °C-25 °C	*Saccharomyces cerevisiae* *Trichophyton rubrum*
6	Robertson's cooked meat[b]	Aerobic 30 °C-35 °C	*Clostridium sporogenes*

Organisms recommended in pharmacopoeias may also be used for growth promotion tests.

[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 may 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 organisms per inoculum (a batch should be regarded as a single preparation from one lot of raw materials autoclaved at one time). Testing should be done with defined strains from recognized type culture collection.

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

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

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

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.

A.6 Process definition [Clause 8]

A.6.1 [8.1] The process definition is undertaken to define the process parameters for a sterilization process, which will achieve the specified requirements for sterility for a defined 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 may 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 may consist of a number of elements, as described in lethal rate screening and studies in 5.3 and Clause 8 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 defined. The appropriateness of this sterilization process with the product load is confirmed in the PQ studies.

A.6.2 [8.2] The stages of sterilizing agent characterization through microbiological process definition may 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 survival curves, and assessment of inactivation of the identified reference microorganism(s) on tissue carriers (see 5.3 and 8.2).

A.6.2.1 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.

A.6.2.2 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.

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 may be capable of surviving the chemical process solutions (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 may include, but is not limited to, process solutions, work surfaces, water purification systems, raw materials and personnel. See Table A.2 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 qualification and validation studies. The relative resistance of the challenge microbiological isolates should be evaluated with regard to the overall sterilization process.

A.6.2.3 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 containing at least 10^5 cells of an isolate to the 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 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.

A.6.2.4 Examples of microorganisms which have been employed for characterization are given in Table A.2. 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.

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 Clause 8, and those representing potential bioburden which might possess some resistance to the process chemicals, including the following (see A.6.2 for guidance on selected reference microorganism):

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

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

c) 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;

d) 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;

e) a range of microbiological species.

Table A.2 — Examples of microorganisms which have been used for assessment of the activity of specific liquid chemical sterilizing agents

Species	Culture collection no.		
	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 As an alternative, similar or equivalent cultures 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.6.3 [8.2.2] 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 may occur due to dilution or reaction with the product. Temperature may increase (or decrease) as a result of heat transfer. Hence, the least lethal temperature might be observed at the start of the process, and the least lethal chemical concentration might be observed at the end.

A.6.4 [8.2.3] 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.6.5 [8.2.4] 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 establishing 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 establishing viable microorganisms on the carrier prior to exposure to the 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 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 might confer resistance, provision should be made to account for any wash-off of the test organism inoculum that might occur during the testing for development of the inactivation curve or MPN data. In order to account for reduction in inoculum, it could be desirable to determine the proportion of the inoculum that might wash off the intended inoculum site.

Cultivation of microorganism(s) in simulated manufacturing conditions should be utilized for microbes which might co-exist with the material during storage in the manufacturing process solutions. This might 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 established. Under such conditions, the level of viable microorganisms present in and/or on the product must 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 defined and validated. The validation should demonstrate that the method chosen recovers the surviving organisms in a reproducible manner.

Test methods may be conducted by either direct enumeration to establish the number of survivors over time (survivor curve) or by Most Probable Number (MPN) estimation (e.g. Stumbo, Murphy, Cochran or Spearman Karber methods); see Reference [18]. Survivor curve construction is preferred, as this method establishes 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 a survivor 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.6.6 [8.2.5] As a conservative approach, the equation for calculation of exposure time in 8.2.5 includes an additional safety factor of 100 to the estimate of the bioburden to compensate for these limitations.

A.6.7 [8.3] 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.7 Validation [Clause 9]

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.

A.8 Routine monitoring and control [Clause 10]

A.8.1 [10.2] See A.5.3 and A.6.2 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.8.2 [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 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 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 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, sterilizing agent solutions are filtered prior to use in order to remove any microorganisms and other impurities carried over from the components of the 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 sterilizing agent post-sterilization should be checked after the product has been removed. The chemical composition of the chemical sterilizing agent must 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 sterilizing agent to demonstrate continued microbicidal efficacy. The 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 chemical sterilizing agent may 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 guidance on aseptic transfer, if applied, see ISO 13408-1.

A.8.3 [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.

A.8.4 [10.5] The sterilizing agent may be collected from at least one of the following:

a) a sample from the product batch;

b) items designed to be representative of the batch, and which are sterilized along with the batch (e.g. scrapped surrogate product).

Final product testing is generally of limited value in determining sterility of a batch of product. However, in this particular application, certain gross failures may be detected by testing for microbiological contamination after completion of the process. When conducting such microbiological tests, it is important to ensure the removal of any residual microbicidally active sterilizing agent or storage solution.

Culture media and incubation conditions must be qualified to support growth of microorganisms similar to those that potentially are in the bioburden. Culture media and conditions may be chosen for the tests for viable microorganisms that are different from those used for a pharmacopoeial test for sterility. However, a pharmacopoeial test for sterility is required for the product that is aseptically packaged after sterilization as specified in 10.5, in accordance with applicable local, national and international requirements.

A.9 Product release from sterilization [Clause 11]

A.9.1 [11.1.1] If a sterilization process operating within specified tolerances has been demonstrated to be both effective and reproducible, confirmation that the process parameters were within specification limits is taken as evidence of the adequacy of the process.

Parametric release is the declaration of adequacy of sterilization of product based on the direct measurement and evaluation of process parameters. No batch sample or indicator microbiological testing is required for parametric release (see ISO 14937). For parametric release, data should be gathered for additional process parameters.

The appropriateness of parametric release should be demonstrated during the validation of the sterilization process. Prior to implementation of parametric release as described in Clause 11, it is necessary to demonstrate a history of acceptable results in the final product test for viable microorganisms, and also that, historically, bioburden has not been excessively resistant to the chemical sterilizing agent. In addition, key process parameters, including post-chemical test results, should fall within the specified tolerances over a history of processing. Parametric release should be supported by extensive experience with the sterilization process.

A.9.2 [11.2] Failure to meet the process specification or failure of an indicator to meet its specified requirements should lead to the affected product being placed in quarantine and the cause of failure investigated. The investigations should be documented and recorded.