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Sterilization of medical devices — Microbiological methods —

Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

Stérilisation des dispositifs médicaux — Méthodes microbiologiques —

Partie 2: Essais de stérilité pratiqués au moment de la définition, de la validation et de la maintenance d'un procédé de stérilisation

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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 11737-2 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 11737-2:1998) which has been technically revised.

ISO 11737 consists of the following parts, under the general title *Sterilization of medical devices — Microbiological methods*:

— *Part 1: Determination of a population of microorganisms on products*

— *Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process*

Introduction

A sterile medical device is one that is free from viable microorganisms. International Standards that 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 from all sources 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. Such products are non-sterile. The purpose of sterilization is to inactivate the microbiological contaminants and thereby transform the non-sterile products 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 item in a population 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 product item.

Generic requirements of the quality management system for design and development, production, installation and servicing are given in ISO 9001[16] and particular requirements for quality management systems for medical device production are given in ISO 13485. The standards for quality management systems recognise 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.

International Standards specifying procedures for the development, validation and routine control of the processes used for sterilization of medical devices have been prepared (see ISO 11135-1[1], ISO 11137-1[3], ISO 14937[12], ISO 14160[7], ISO 17665-1[13] and ISO 20857[14]). An element of validation might consist of exposing medical devices to the sterilizing agent with the extent of treatment being reduced relative to that which will be used in routine sterilization processing, in order to provide a knowledge of the resistance to the agent of the microbial contamination as it occurs naturally on medical devices. Subsequent to this exposure, medical devices are subjected individually to tests of sterility as described in this part of ISO 11737. Examples of the use of such tests are in a) establishing a dose for sterilization by radiation, and b) demonstrating the continued validity of an established sterilization dose.

Annex A of this part of ISO 11737 gives guidance on the techniques used and on practical aspects of the requirements.

Sterilization of medical devices — Microbiological methods —

Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

1 Scope

1.1 This part of ISO 11737 specifies the general criteria for tests of sterility on medical devices that have been exposed to a treatment with the sterilizing agent reduced relative to that anticipated to be used in routine sterilization processing. These tests are intended to be performed when defining, validating or maintaining a sterilization process.

1.2 This part of ISO 11737 is not applicable to:

a) sterility testing for routine release of product that has been subjected to a sterilization process;

b) performing a test for sterility (see 3.12);

NOTE 1 The performance of a) or b) is not a requirement of ISO 11135-1, ISO 11137-1, ISO 14160, ISO 14937 or ISO 17665-1.

c) culturing of biological indicators or inoculated products.

NOTE 2 Guidance on culturing biological indicators is included in ISO 14161[8].

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, *Measurement management systems — Requirements for measurement processes and measuring equipment*

ISO 11737-1:2006, *Sterilization of medical devices — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

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

ISO/IEC 17025:2005, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

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

3.1
aerobic organism
microorganism that requires oxygen for metabolism

3.2
anaerobic organism
microorganism that does not require oxygen for metabolism

3.3
bacteriostasis/fungistasis test
technical operation performed with selected microorganisms to detect the presence of substances that inhibit the multiplication of these microorganisms in a test of sterility

3.4
bioburden
population of viable microorganisms on a product and/or a package

NOTE Adapted from ISO/TS 11139:2006, definition 2.2.

3.5
culture conditions
combination of growth media and manner of incubation used to promote germination, growth and/or multiplication of microorganisms

NOTE The manner of incubation can include the temperature, time and any other conditions specified for incubation.

[ISO/TS 11139:2006, definition 2.10]

3.6
facultative organism
microorganism capable of both aerobic and anaerobic metabolism

3.7
growth promotion test
technical operation performed to demonstrate that a growth medium will support microbial multiplication

3.8
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 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 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 has been developed by the Global Harmonization Task Force (GHTF 2002).

3.9
product
result of a process

NOTE 1 For the purposes of sterilization standards, product is tangible and can be raw material(s), intermediate(s), sub-assembly(ies) and health care product(s).

NOTE 2 Adapted from ISO 9000:2005, definition 3.4.2.

3.10
sample item portion
SIP
defined part of a medical device that is tested

3.11
sterile
free from viable microorganisms

[ISO/TS 11139:2006, definition 2.43]

3.12
test for sterility
technical operation defined in a Pharmacopoeia, performed on product following exposure to a sterilization process

[ISO/TS 11139:2006, definition 2.53]

3.13
test of sterility
technical operation performed as part of development, validation or requalification to determine the presence or absence of viable microorganisms on product or portions thereof

[ISO/TS 11139:2006, definition 2.54]

4 Quality management system elements

4.1 Documentation

4.1.1 Procedures for the performance of tests of sterility shall be specified.

4.1.2 Documents and records required by this part of ISO 11737 shall be reviewed and approved by designated personnel (see 4.2.1). Documents and records shall be controlled in accordance with ISO 13485 or ISO/IEC 17025. Records retained shall include all original observations, calculations, derived data and final reports. The records shall include the identity of all personnel involved in sampling, preparation and testing.

4.1.3 Calculations and data transfers shall be subjected to appropriate verification.

4.2 Management responsibility

4.2.1 The responsibility and authority for implementing and performing the procedures described in this part of ISO 11737 shall be specified. Responsibility shall be assigned to competent personnel in accordance with ISO 13485 or ISO/IEC 17025.

4.2.2 If the requirements of this part of ISO 11737 are undertaken by organizations with separate quality management systems, the responsibility and authority of each party shall be specified.

4.2.3 All equipment required for correct performance of the specified tests and measurements shall be available.

4.3 Product realization

4.3.1 Procedures for purchasing shall be specified. These procedures shall comply with ISO 13485 or ISO/IEC 17025.

4.3.2 A documented system complying with ISO 13485, ISO/IEC 17025 or ISO 10012 shall be specified for the calibration of all equipment, including instrumentation for test purposes, used in meeting the requirements of this part of ISO 11737.

4.3.3 Equipment or parts thereof that come into contact with product, eluent or media during testing shall be sterile.

4.3.4 Methods shall be specified for the preparation and sterilization of materials used in the tests of sterility, including appropriate quality tests.

4.4 Measurement, analysis and improvement

Procedures for investigation of unusual, unexpected or out-of-specification results and for correction, corrective action and preventive action shall be specified. These procedures shall comply with ISO 13485 or ISO/IEC 17025.

5 Selection of product

5.1 General

5.1.1 The procedures for selection and handling of product for performing tests of sterility shall ensure that product is representative of routine production, including packaging materials and processes. (See also 5.3.)

5.1.2 If product is grouped for the purposes of development, validation and routine control of the sterilization process in which tests of sterility are performed, the rationale for inclusion of a product within a group shall be recorded (see 4.1.2). The rationale shall include criteria to ensure that product selected for testing is representative of the whole group.

5.2 Sample item portion (SIP)

5.2.1 If allowed in an applicable standard, for the development, validation and routine control of the sterilization process, and when the use of an entire product is not practicable, a selected portion of product [sample item portion (SIP)] may be substituted if allowed by the sterilization method.

5.2.2 If the bioburden distribution on/in product, is not known, the SIP shall consist of portions of product selected at random, which proportionally represent each of the materials from which product is made.

If the bioburden distribution is known and bioburden is evenly distributed on/in product, the SIP may be selected from any portion of the product.

If the bioburden distribution is known and bioburden is not evenly distributed on/in product, the SIP shall either be selected from the portion of product that is considered to be the most severe challenge to the sterilization process or consist of portions of product, selected at random, which proportionally represent each of the materials from which product is made.

5.2.3 The adequacy of a selected SIP shall be demonstrated.

NOTE The standard specifying requirements for development, validation and routine control of the sterilization process might stipulate the criteria for the adequacy of the SIP.

5.3 Packaging of product and sample item portions

If packaging materials and/or methods for product or SIPs to be used in tests of sterility are different from those used in routine production, selection of packaging material and the method of packaging shall ensure that:

a) product or SIP receives the intended treatment with the sterilizing agent;

b) microbiological status of product or SIP is maintained;

c) access of the sterilizing agent to product or SIP is similar to that achieved with packaging used in routine production.

6 Methods for performing tests of sterility

6.1 There are two general methods for performing tests of sterility. These are:

a) direct immersion of product in growth medium or addition of growth medium in product, followed by incubation;

b) removal of microorganisms from product and transfer of removed microorganisms to growth medium followed by incubation.

6.2 For an identified product, factors that influence the design of the method for performing tests of sterility shall be considered and recorded (see 4.1.2). Factors to be considered include, at least:

a) the part(s) of product for which sterility is claimed on the label;

b) the physical and/or chemical nature of product to be tested (see also 6.6);

c) possible type(s) of contaminating microorganisms and their locations on/in product.

6.3 In performing tests of sterility, aseptic technique shall be applied in carrying out manipulations that might affect the result of the test.

6.4 If microorganisms are to be removed from product by elution before transfer to growth medium, factors to be considered shall include:

a) selection of an appropriate eluent;

b) ability of the elution technique to remove contaminating microorganisms effectively (see, for example, 7.2 of ISO 11737-1);

c) effect(s) of the elution technique on the viability of contaminating microorganisms.

6.5 If microorganisms are to be removed from an eluent or a fluid product by filtration before transfer to growth medium, factors to be considered shall also include:

a) selection of an effective filtration system;

b) selection of an appropriate fluid for rinsing the container, the filter and associated equipment (if needed).

6.6 If the physical or chemical nature of product to be tested [see 6.2 b)] is such that substances might be present or released which could adversely affect the multiplication of microorganisms, a system to neutralize, remove, or, if this is not possible, minimize the effect of any such substances shall be used. The effectiveness of such a system shall be demonstrated.

6.7 Culture conditions shall be selected after consideration of the types of microorganisms expected to be present. The results of this consideration and the rationale for the decisions reached shall be recorded (see 4.1.2).

6.8 The interval of time between exposure of product to the sterilizing agent and performing tests of sterility on such product shall be as short as practicable.

6.9 Following incubation, the growth medium shall be examined for evidence of microbial growth and the results of this examination shall be recorded (see 4.1.2).

7 Assessment of method for performing tests of sterility

Prior to utilizing the outcomes from tests of sterility, the appropriateness of the selected method shall be assessed and the results of the assessment shall be recorded (see 4.1.2).

8 Maintenance of the method for performing tests of sterility

8.1 Modifications to product and/or manufacturing process shall be reviewed to determine whether they are likely to require a change in the method for performing tests of sterility. If the review indicates that a change is needed, the requirements given in Clause 6 shall apply.

8.2 Modifications to the method for performing tests of sterility shall be assessed to determine their effect on the continued appropriateness of the test method. The results of this assessment shall be recorded (see 4.1.2).

Annex A
(informative)

Guidance on tests of sterility performed in validation and maintenance of a sterilization process

A.1 Scope

This annex contains guidance on the implementation of the requirements specified in this part of ISO 11737. The guidance given is not intended to be exhaustive, but to highlight important aspects to which attention should be given.

Methods other than those given in this annex may be used, but such alternative methods should be demonstrated as being effective in achieving compliance with the requirements of this part of ISO 11737.

This annex is not intended as a checklist for assessing compliance with the requirements of this part of ISO 11737.

A.2 Normative references

The requirements specified in documents included as normative references are requirements given in this part of ISO 11737 only to the extent that they are cited in a normative part of this part of ISO 11737; the citation may be to an entire standard or limited to specific clauses.

In regard to the normative reference to ISO 13485, it should be noted that it is not a requirement of this part of ISO 11737 to have a full quality management system. However, the elements of a quality management system which are the minimum necessary to control the tests of sterility used to validate and maintain the sterilization process for medical devices are normatively referenced at appropriate places in the text (see, in particular, Clause 4). Attention is drawn to the standards for quality management systems for all stages of production or reprocessing of medical devices (see ISO 13485) and for laboratory quality management systems (see ISO/IEC 17025). National and/or regional regulations for the provision of medical devices might require the implementation of a complete quality management system and the assessment of that system by a third party.

A.3 Terms and definitions

No guidance offered.

A.4 Quality management system elements

A.4.1 Documentation

A.4.1.1 No guidance offered.

A.4.1.2 In ISO 13485, the requirements in the documentation section relate to the generation and control of documents (including specifications and procedures) and records.

Requirements for control of documents and records are specified in 4.2.3 and 4.2.4 of ISO 13485:2003, or 4.3, 4.13 and 5.4 of ISO/IEC 17025:2005.

A.4.1.3 Computers might be used in laboratories for direct and indirect collection, processing and/or storage of data. Both the hardware and software used for such applications should be controlled.

The computer system in use should be identified, both in terms of hardware and software, and any changes in either of these should be documented and subject to approval (see 4.1.2 and 4.2.1).

If calculations are performed by electronic data processing techniques, the software (e.g. spreadsheet calculations) should be validated prior to use and records of this validation should be retained.

For software, there should be documentation describing:

— applications software;

— operations software;

— data packages in use.

All software should be acceptance tested before being put into service.

If computer software is developed in-house, suitable procedures should be developed to ensure that:

— documentation describing development, including the source code, is retained;

— records of acceptance testing are retained;

— modifications to programs are documented;

— changes to equipment are documented and changed equipment is tested before being put into use.

These controls should be applied to any modification or customizing of commercial software packages.

There should be procedures to detect or prevent unauthorized changes to software.

Software which organizes, tabulates and/or subjects data to statistical or other mathematical procedures, or which otherwise manipulates or analyses the electronically stored data, should permit retrieval of original data entries. Special procedures for archiving computer data are likely to be required and these procedures should be documented.

See also ISO 90003[17] for guidance of the application of quality management systems to computer software.

A.4.2 Management responsibility

A.4.2.1 In ISO 13485, the requirements in the management responsibility section relate to management commitment, customer focus, quality policy, planning, provision of resources, responsibility, authority and communication, and management review.

Laboratories should be committed to providing a quality service and this commitment should be documented as a quality policy. The lines of responsibility and authority within the laboratory organization should be defined and documented. An individual should be nominated to be responsible for the laboratory quality management system and should have the authority to ensure that the system is implemented.

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

A.4.2.2 The use and performance of tests of sterility can involve separate parties, each of whom is responsible for certain elements of the method or procedure. This part of ISO 11737 requires that the party accepting particular responsibilities be defined and that this definition of responsibilities be documented. This definition of responsibility is documented within the quality management system(s) of the identified parties. The party accepting responsibilities for defined elements is required to assign these elements to competent personnel, with competence demonstrated through appropriate training and qualifications.

If tests of sterility are performed in a laboratory under the direct management of the manufacturer of the medical device, the operation of the laboratory resides within the manufacturer's quality management system. If an external laboratory is used, the laboratory should either be certified against an appropriate International Standard (e.g. ISO/IEC 17025) or applicable regulatory requirements.

A.4.2.3 Requirements for provision of resources are specified in Clause 6 of ISO 13485:2003 and requirements for equipment are specified in 5.5 of ISO/IEC 17025:2005.

A.4.3 Product realization

A.4.3.1 In ISO 13485, the requirements in the product realization section relate to the product lifecycle from the determination of customer requirements, design and development, purchasing, control of production, and calibration of monitoring and measuring devices.

Requirements for purchasing are specified in 7.4 of ISO 13485:2003. In particular, it should be noted that the requirements in 7.4.3 of ISO 13485:2003 for verification of purchased product, apply to all products and services received from outside the organization.

A.4.3.2 There should be a system for identifying the maintenance requirements for each piece of laboratory equipment.

Equipment that does not require calibration should be clearly identified.

Requirements for calibration of monitoring and measuring devices are specified in 7.6 of ISO 13485:2003. Requirements for equipment and measurement traceability requirements are specified in 5.5 and 5.6 of ISO/IEC 17025:2005.

A.4.3.3 Eluents or media used for the removal of microorganisms should be prepared in a manner that ensures their sterility.

A.4.3.4 Appropriate quality tests should include growth promotion tests. Generally, growth promotion tests are performed on each batch of medium using an inoculum of low numbers (between 10 and 100 colony forming units) of selected microorganisms. Growth promotion tests are described in pharmacopoeial monographs which detail suitable microorganisms.

A.4.4 Measurement, analysis and improvement

In ISO 13485, the requirements in the measurement, analysis and improvement section relate to in-process monitoring, analysis of data and improvement (including corrective and preventive actions).

The operation of the laboratory should be subject to regular internal audits. The results of audits should be documented and reviewed by the laboratory management.

Unusual, unexpected or out-of-specification results from the performance of tests of sterility require investigation. The initial phase of the investigation should involve assessing if the results are a true finding or are in error. The following can contribute to an error and should be addressed:

— inappropriate samples (e.g. non-representative, non-homogeneous or rejected materials);

— unsuitable conditions of transport/handling/storage;

— inappropriate test materials (e.g. pipettes, filtration apparatus);

— incorrect handling or test method(s);

— inappropriate media or diluents;

— inappropriate laboratory environment;

— inappropriate incubation environment;

— errors of interpretation of the test result;

— errors of transcription.

Based on the results of the investigation, specific corrective action might be required. If corrective action is required, its effectiveness has to be demonstrated.

Procedures for corrective action are specified in 8.5.2 of ISO 13485:2003 and 4.11 of ISO/IEC 17025:2005.

A.5 Selection of product

A.5.1 General

A.5.1.1 Product is chosen from a batch of product produced under conditions that are representative of routine processing procedures. It is preferred to select product for testing at random.

The number of product items that are selected and the number of batches from which this selection is made might be described in the relevant International Standard specifying the requirements for validation and routine control of the sterilization process.

Techniques for selecting and handling samples of product should be chosen and performed to avoid the introduction of inadvertent contamination and alterations to the numbers and types of microorganisms on/in the sample.

Product for testing may be selected from items rejected during the manufacturing process provided that they have been subjected to the same processing and conditions applied to acceptable items and that the cause behind rejection does not compromise the validity of the test.

A.5.1.2 Requirements relating to the grouping of products are generally described in the particular International Standard for development, validation and routine control of the sterilization process (see, for example, ISO 11135-1[1] and ISO 11137-2[4]).

A.5.2 Sample item portion (SIP)

A.5.2.1 Whenever it is practical, an entire product item should be used for testing, but it is recognized that this is not always possible. In such situations, a selected portion of a product (SIP), which is convenient to handle during testing, may be substituted. The SIP should be as large a portion of the product as is possible to manipulate readily in the laboratory. The SIP can be selected on the basis of length, mass, volume or surface area of the product. See Table A.1.

Table A.1 — Examples for selection of SIP

Basis for SIP	Product
Length	Tubing (consistent diameter) Rolls of bandage
Mass	Powders Gowns
Volume	Liquids
Surface area	Surgical drapes Tubing (variable diameter)

If a product or SIP cannot be tested in available laboratory containers, it may be divided into two or more containers and these containers scored together as one; if one container yields a positive result, the entire product item is considered positive.

If the product item has a label claim of sterility of the fluid path only, the fluid path should be regarded as the entire product item (i.e. SIP = 1).

A.5.2.2 The microbial contamination on the SIP shall represent the microbiological challenge presented to the sterilization process. If the product is complex, the SIP shall represent the bioburden of the diverse elements of the product.

A.5.2.3 No guidance provided.

A.5.3 Packaging of product and sample item portions

It is preferred that product be exposed to the sterilizing agent in its original form and package. However, to minimize and/or simplify the manipulations in performing tests of sterility and thereby reduce the possibility of false positives arising from contamination, product may be disassembled and repackaged prior to exposure to the sterilizing agent.

It is important to consider the effect of disassembling and repackaging of product on the response of the microorganisms to the sterilizing agent. For example, disassembling might alter the chemical environment of the microorganisms.

It is also important to consider the effect of disassembling the product on the access of the sterilizing agent to the microorganisms.

If the SIP is prepared and packaged prior to the exposure to the sterilizing agent, this should be conducted under conditions chosen to minimize alteration of the bioburden.

A.6 Methods for performing tests of sterility

A.6.1 As indicated in Clause 6, the method of performing tests of sterility can be broadly divided into two general categories as described in a) and b).

a) *Direct immersion of product*: direct immersion is the preferred method of performing tests of sterility on medical devices. With direct immersion, the product or SIP is placed aseptically in a container (or multiple containers, see A.5.2.1) of growth medium and then incubated. A sufficient amount of growth medium should be used to achieve contact between the growth medium and the whole of the product or SIP. Additionally, consideration should be given to:

   — disassembly prior to exposure to the sterilizing agent (see also A.5.3);

   — disassembly and/or manipulation prior to immersion in the growth medium;

   — agitation after placement in growth medium;

   — the addition of a surfactant (which has been demonstrated to have no microbiostatic or microbicidal effect) to the growth medium in order to improve a moistening of the product surface.

   Contact should be maintained between the growth medium and the product or SIP for the duration of the incubation period.

   For the performance of a test of sterility on the fluid path of a product, the fluid path is filled with growth medium and the product is incubated.

b) *Removal of microorganisms from product*: when it is not possible to use direct immersion due to characteristics of the medical device, such as bacteriostatic/fungistatic activity, removal of microorganisms might be necessary.

Care should be exercised in using this technique. The technique might not elute all microorganisms from product. An inability to remove all microorganisms from product can result in the test being invalid. Contamination occurring during associated manipulations can result in the occurrence of false positives.

Procedures in which microorganisms are removed from the product by physical treatment before transfer to culture conditions can, in turn, be further subdivided into:

— elution and membrane filtration;

— elution and culturing of the eluate.

In both these subdivisions, the initial action is to remove microorganisms from the product or SIP. The techniques employed are the same as those used in bioburden determination and have been described in B.2.2 of ISO 11737-1:2006. Similarly, the considerations for selecting a suitable eluent are the same as for the bioburden determination and have been described in B.2.3 and Table B.1 of ISO 11737-1:2006.

Once the microorganisms have been removed from the product item or SIP, the test of sterility may be performed using membrane filtration or culturing of the entire eluate (see A.6.4.)

A.6.2 No guidance provided.

A.6.3 Aspects of aseptic technique applicable in performing tests of sterility include the following.

— Conducting the test in a controlled environment.

EXAMPLES Laminar flow hood or biosafety cabinet located in a dedicated, environmentally controlled room; barrier isolation.

NOTE Further information on controlled environment is given in ISO 14644-1[9], 14644-4[10] and 14644-7[11].

— Sterilizing all equipment, materials and items used in the test.

— Introducing the test utensils, growth media and test articles into the test area aseptically.

— Decontaminating the package exterior prior to introduction of the test articles into the test area.

— Decontaminating the surfaces in the test area.

— Minimizing the manipulations required to perform the test.

— Training in the performance of aseptic techniques.

A.6.4 To perform tests of sterility by culturing the eluate, one approach is to use growth medium as the eluent and, after elution, to transfer the eluate to sterile containers and then incubate.

Another approach is to use an eluent that does not support microbial growth and, after elution, the eluate is mixed with an equal volume of double-strength growth medium in sterile containers and incubated. Alternatively, if the volume of the eluate is not more than 10 % of the volume of the growth medium, the eluate can be mixed with normal strength growth medium in sterile containers and incubated.

A.6.5 To perform tests of sterility using filtration, the eluate is passed through a sterile membrane filter with a nominal pore size not greater than 0,45 µm with the aid of vacuum or pressure.

Surfaces that have been in contact with eluate are rinsed with further sterile eluent or solution containing a neutralizer (see A.6.6), and the rinsing solution is passed through the membrane filter. Thereafter, either the

growth medium is transferred aseptically to the filtration unit or the membrane filter is transferred aseptically to growth medium.

Both of these operations are followed by incubation.

A.6.6 Product being tested should be screened to determine if any inhibitory substances are released into the medium which can cause a false negative (see A.7). This is performed by the inoculation of low numbers of representative organisms into the medium containing a product as is carried out in the bacteriostasis/fungistasis test.

If microbicidal or microbiostatic substances are detected, their influence can be minimized by:

a) addition of neutralizer(s) to the growth medium or eluent;

b) removal of the microbicidal or microbiostatic substance from an eluate by filtration;

c) reduction of the concentration of the microbicidal or microbiostatic substance to an ineffective level by dilution. This may be achieved by increasing the volume of growth medium or eluent and, where necessary, subdividing the product into a number of test containers.

Generally, the procedures, organisms, titers and incubation times referenced in current Pharmacopeias are appropriate, but the incubation temperature(s) and medium (media) have to be the same as those to be used in performing tests of sterility.

A.6.7 The particular International Standard for development, validation and routine control of the sterilization process might recommend the culture conditions to be employed in the test of sterility.

Generally, one type of culture growth medium is used on the assumption that it will be optimal for the culturing of aerobic and facultative microorganisms which might survive exposure to the sterilizing agent. When using Soybean-Casein Digest Medium as the only growth medium, culture conditions of 30 ± 2 °C for 14 d are commonly employed. When another growth medium is used in performing tests of sterility, other incubation conditions should be considered.

The incubation temperature recommended for tests of sterility might be lower than that recommended for determination of bioburden. The use of the lower temperature and longer incubation times can aid the recovery of damaged or injured microorganisms.

A choice of culture conditions will need to be made if:

— the particular International Standard for development, validation and routine control of the sterilization process does not stipulate the growth medium to be used;

— the use of a single set of culture conditions is not appropriate because of the types of microorganism likely to survive exposure to the sterilizing agent (e.g. the presence of anaerobes or mycobacteria).

Factors to be considered in choosing culture conditions in these instances should include the following:

— the nature of the product;

— the method of manufacture;

— the sources of potential microbiological contamination;

— the types of microorganism likely to be encountered.

Information about the types of microorganism from bioburden determinations performed in accordance with ISO 11737-1 might assist in the selection of culture conditions.