INTERNATIONAL STANDARD

ISO 11737-2

Third edition
2019-12

Sterilization of health care products — Microbiological methods —

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

Stérilisation des produits de santé — Méthodes microbiologiques —

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

Reference number
ISO 11737-2:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

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

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

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

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

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

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

This third edition cancels and replaces the second edition (ISO 11737-2:2009), which has been technically revised.

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

— addition of a requirement concerning the test samples and the interval of time between the manufacture of product and the exposure to the sterilizing agent being as short as possible;

— addition of a requirement about the samples staying immersed in the culture media and providing a rationale where this is not possible;

— provision of additional guidance regarding performing tests of sterility on packaging, clarifying that package testing is not typically done except when it is an integral part of the product;

— provision of additional guidance regarding what is meant by “controlled environment” for performing tests of sterility;

— provision of additional guidance to discuss circumstances where the method suitability test does not give acceptable results, stating that after multiple attempts to eliminate inhibitory substances, it is appropriate to accept a reduction of inhibitory substances, with an accompanying rationale and risk assessment;

— provision of guidance regarding identification of microbial growth in a test of sterility, saying generally for positive growth the microorganism(s) should be identified;

— provision of guidance regarding method suitability, saying that consideration should be given to periodically demonstrating ongoing method suitability in order to ensure that an accumulation of minor changes over time has not occurred;

— addition of a table to clarify where typical responsibilities reside for the manufacturer or the laboratory.

A list of all parts in the ISO 11737 series can be found on the ISO website.

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

Introduction

A sterile medical device is one that is free 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) can, prior to sterilization, have microorganisms on them. 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 might 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 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, ISO 11137 (all parts), ISO 14937, ISO 14160, ISO 17665-1 and ISO 20857]. 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. The reduced exposures applied in these instances are often called fractional exposures or verification doses. Subsequent to this reduced exposure, medical devices are subjected individually to tests of sterility as described in this document. Examples of the use of such tests are in:

a) establishing a dose for sterilization by radiation,

b) demonstrating the continued validity of an established sterilization dose, and

c) establishing a cycle for sterilization by evaluating the product's naturally occurring bioburden.

Product that has been exposed to a terminal sterilization process in its final packaged form has a very low probability of the presence of a viable microorganism; such as one in one million or 10^{-6}. As such, performing a test of sterility on product that has been exposed to the complete sterilization process provides no scientifically usable data and is not recommended.

Annex A of this document gives guidance on the techniques used and on practical aspects of the requirements.

Sterilization of health care products — Microbiological methods —

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

1 Scope

1.1 This document specifies the general criteria for tests of sterility on medical devices that have been exposed to a treatment with the sterilizing agent which has been 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 document 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, ISO 11137-1, ISO 11137-2, ISO 14160, ISO 14937, ISO 17665-1 or ISO 20857.

c) test of sterility or test for sterility for demonstration of product shelf life, stability and/or package integrity, and

d) culturing of biological indicators or inoculated products.

NOTE 2 Guidance on culturing biological indicators is included in ISO 11138-7.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
aseptic technique
conditions and procedures used to minimize the risk of the introduction of microbial contamination

[SOURCE: ISO 11139:2018, 3.16]

3.2
bacteriostasis/fungistasis test
technical operation performed to detect the presence of substances that inhibit microbial multiplication

[SOURCE: ISO 11139:2018, 3.20]

3.3
bioburden
population of viable microorganisms on or in a product and/or sterile barrier system

[SOURCE: ISO 11139:2018, 3.23]

3.4
culture condition
combination of growth media and manner of incubation used to promote germination, growth and/or multiplication of microorganisms

Note 1 to entry: The manner of incubation can include the temperature, time, and any other conditions specified for incubation.

[SOURCE: ISO 11139:2018, 3.70]

3.5
facultative organism
microorganism capable of both aerobic and anaerobic metabolism

[SOURCE: ISO 11139:2018, 3.114]

3.6
health care product
medical device (3.7), including in vitro diagnostic medical device, or medicinal product, including biopharmaceutical

[SOURCE: ISO 11139:2018, 3.132]

3.7
medical device
instrument, apparatus, implement, machine, appliance, implant, reagent for in vitro use, or software material or other similar or related article, intended by the manufacturer to be used, alone or in combination, for human beings, for one or more of the specific medical purpose(s) of:

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

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

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

— supporting or sustaining life;

— control of conception;

— disinfection of medical devices;

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

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

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

— items specifically intended for cleaning or sterilization of medical devices;

— pouches, reel goods, sterilization wrap, and reusable containers for packaging of medical devices for sterilization;

— disinfection substances;

— aids for persons with disabilities;

— devices incorporating animal and/or human tissues;

— devices for in vitro fertilization or assisted reproduction technologies.

[SOURCE: ISO 13485:2016, 3.11, modified — The first two list items in the Note 1 to entry have been added.]

3.8
method suitability
<microbiological> assessment of the test method to demonstrate its ability to allow microbial growth

[SOURCE: ISO 11139:2018, 3.168]

3.9
product
tangible result of a process

EXAMPLE Raw material(s), intermediate(s), sub-assembly(ies), health care product(s).

[SOURCE: ISO 11139:2018, 3.217]

3.10
sample item portion
SIP
specified part of a health care product that is tested

[SOURCE: ISO 11139:2018, 3.240, modified — Acronym SIP has been added.]

3.11
sterile
free from viable microorganisms

[SOURCE: ISO 11139:2018, 3.271]

3.12
test for sterility
technical operation specified in a pharmacopoeia performed on product following an aseptic process or exposure to a sterilization process

[SOURCE: ISO 11139:2018, 3.298]

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

Note 1 to entry: This is performed after exposure to the sterilizing agent at a level which is reduced compared to the complete sterilization process.

[SOURCE: ISO 11139:2018, 3.299, modified — Note 1 to entry added.]

4 General

4.1 The development, validation and routine control of a sterilization process is a critical element in product realization of health care products. To ensure the consistent implementation of the requirements specified in this document, the necessary processes need to be established, implemented

and maintained (see Annex B). Processes of particular importance in relation to the development, validation and routine control of a sterilization process include but are not limited to:

— control of documentation, including records;

— assignment of management responsibility;

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

— control of product provided by external parties;

— identification and traceability of product throughout the process; and

— control of non-conforming product.

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

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

5 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 the selected product is representative of routine production, including packaging materials and processes (see also 5.3).

5.1.2 If product(s) is grouped in a product family 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 product family shall be recorded. The rationale shall include criteria to ensure that a product selected from a product family for testing is representative of the whole product family.

5.1.3 The rationale for the number of product items that are selected and the number of batches from which this selection is made shall be documented.

NOTE This could be described in the relevant International Standard specifying the requirements for validation and routine control of the sterilization process.

5.2 Sample item portion (SIP)

5.2.1 Whenever practicable the test of sterility is performed on the entire product. A selected portion of the product [sample item portion (SIP)] may be substituted for the entire product in the test of sterility when permitted in the applicable sterilization standard.

5.2.2 The determination of portions selected for tests of sterility shall be based on whether the bioburden is known to be evenly distributed (see 5.2.2.1) or not (see 5.2.2.2).

5.2.2.1 When the bioburden distribution is known:

a) if the bioburden is evenly distributed on and/or in the item, the SIP for tests of sterility may be selected from any portion of the item;

b) if the bioburden is not evenly distributed, the SIP for tests of sterility shall include either:

1) portions of product selected that proportionally represent each of the materials from which the product is made, or

2) the portion of the product that contains the most severe microbial challenge (numbers and/or types) to the sterilization process.

When selecting the portion that contains the most severe microbial challenge, the relationship of the bioburden of the SIP tested to the entire product bioburden should be established.

5.2.2.2 If the bioburden distribution is not known, the SIP for tests of sterility shall consist of portions of product selected that proportionally represent each of the materials from which the product is made.

5.2.3 The SIP can be calculated on the basis of dimensional characteristics, such as length, mass, volume or surface area (see Table A.1 for examples).

5.2.4 The adequacy of a selected SIP shall be demonstrated.

NOTE Some standards specifying requirements for validation and routine control of the sterilization process stipulate criteria for the adequacy of the SIP, e.g. ISO 11137-2.

5.3 Packaging of product and sample item portions

It is recommended that packaging for product or SIPs be the same as that used in routine production. If packaging materials and/or processes are different from those used in routine production, it shall be documented. 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 equivalent to that achieved with packaging used in routine production.

6 Methods for performing tests of sterility

6.1 There are three general methods for performing tests of sterility:

a) Direct immersion of product in culture medium or addition of culture medium to product, followed by incubation. The product shall be immersed in the culture media for the duration of the incubation time where possible. A rationale shall be provided if this is not possible, such as with buoyant materials.

NOTE When the term culture medium is used, it means that the culture medium is sterile.

b) Removal of microorganisms from product and transfer of removed microorganisms to culture medium followed by incubation (see 6.4).

c) Filtration of liquid products by immersion of the filter in culture 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. Factors that might be applicable include but are not limited to the following:

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 could affect the result of the test.

6.4 If microorganisms are to be removed from product by elution before transfer to culture medium [see 6.1 b)], factors to be considered shall include:

a) selection of an appropriate eluent;

b) establishment of a recovery efficiency followed by a risk assessment to determine the appropriateness of the removal process (for example, ISO 11737-1:2018, 7.2);

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 culture 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 The test system shall be evaluated in a method suitability test (also called bacteriostasis and fungistasis) to ensure that the ability to sustain microbiological growth is not affected. If the physical or chemical nature of product to be tested [see 6.2 b)] is such that substances could be present or released that 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.

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

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

7 Assessment of the 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.

8 Maintenance of the method for performing tests of sterility

8.1 Modifications to product, or to the processes used to manufacture product, shall be evaluated to determine any possible effect on the ability to detect viable microorganisms in the test of sterility. If the evaluation indicates that a change to the test of sterility is needed, the requirements given in Clauses 5, 6 and 7 shall apply.

8.2 Modifications to the parameters of a test 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.

Annex A
(informative)

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

A.1 Scope

This annex provides guidance on the implementation of the requirements specified in this document. 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 conformity with the requirements of this document.

This annex is not intended as a checklist for assessing conformity with the requirements of this document.

A.1.1 No guidance offered.

A.1.2 Tests for sterility (e.g. sterility testing for lot/batch release) (see 3.12) are excluded from this document because they are not performed in the definition, validation and maintenance of a sterilization process. Tests for sterility are not appropriate for confirmation of sterilization process effectiveness, a sterility assurance level, or attributes associated with the sterility of a product such as package integrity or product shelf life. See also Daniell[24].

A.2 Guidance on normative references

There are no normative references in this document.

It should be noted in particular that it is not a requirement of this document to have a full quality management system. However, there are elements of a quality management system which are applicable to control the tests of sterility used to validate and maintain the sterilization process for medical devices. 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 could require the implementation of a complete quality management system and the assessment of that system by a third party.

A.3 Guidance on terms and definitions

No guidance offered.

A.4 General

A.4.1 No guidance offered.

A.4.2 No guidance offered.

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 production. If product batch size permits, it is preferred to select product for testing at random.

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.

Samples for testing can 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 and ISO 11137-2).

A.5.1.3 No guidance offered.

A.5.2 Guidance on sample item portion (SIP)

A.5.2.1 Whenever practicable, the test of sterility should use the entire product, although this might not be feasible if the product cannot be accommodated in available laboratory testing vessels. In such situations, a selected portion of a product (i.e. SIP), which is convenient to handle during testing, may be substituted. 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 As large a portion of product as possible should be used for the SIP. The microbial bioburden on the SIP should represent the microbiological challenge presented to the sterilization process. If the product is complex, the SIP should represent the bioburden of the diverse elements of the product. Consideration should be given to aspects of manufacturing that contribute to the distribution of microorganisms on product.

Product such as drapes, lengths of tubing, etc. are types of product that could be expected to have an even distribution of bioburden. This might not apply in case of application of manual steps for cutting or folding of drapes as well as for cutting, transportation, and assembly of tubing.

A.5.2.3 Examples of an SIP that can be selected from the device with a more severe challenge to the sterilization process are tubing sets with connections, stopcocks, etc.

Examples of products for which various bases for SIP calculation are employed are given in Table A.1.

Table A.1 — Examples for selection of SIP

Basis for SIP	Product
Surface area	Implants (non-absorbable) Drapes (plastic) Tubing (variable diameter) Rolls of bandage

Table A.1 *(continued)*

Basis for SIP	Product
Mass	Paper Powders Gowns Implants (absorbable) Rolls of bandage
Length	Tubing (consistent diameter) Rolls of bandage
Volume	Fluid in a container

A.5.2.4 No guidance offered.

A.5.3 Packaging of product and sample item portions

It is preferred that product be exposed to the sterilization agent in its original form and packaging. However, to minimize and/or simplify the manipulations in performing tests of sterility and thereby reduce the possibility of false positives arising from contamination not related to the product or production process, 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, e.g. anaerobic environment to aerobic environment.

It is also important to consider the effect of disassembling the product on the access of the sterilizing agent to the microorganisms. For example, disassembling could allow access of the sterilizing agent that is not representative of routine processing.

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 for solid product 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 health care products. With direct immersion, the product or SIP is placed aseptically in a container (or multiple containers, see A.5.2.1) of culture medium and then incubated. A sufficient amount of culture medium should be used to achieve contact between the culture medium and the whole of the product or SIP. Additionally, consideration should be given to:

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

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

   — agitation after placement in culture medium;

   — the addition of a surfactant (which has been demonstrated to have no inhibitory effects, e.g. no microbiostatic or microbicidal effect) to the culture medium in order to improve a moistening of the product surface.

   Contact should be maintained between the culture medium and the product or SIP for the duration of the incubation period, if possible. If this is not possible due to the buoyant nature of the product, a procedure should be implemented to periodically manipulate the container so that contact is facilitated during the incubation period.

For the performance of a test of sterility on the fluid path of a product, the fluid path is filled with culture medium and the product is incubated. For a rinse or flush method of a fluid path, see b).

b) Removal of microorganisms from product: when it is not possible to use direct immersion due to characteristics of the health care product, such as size or bacteriostatic/fungistatic activity, removal of microorganisms could be necessary.

Care should be exercised in using this technique as elution of microorganisms from product is often not as effective for a test of sterility compared to direct immersion. Therefore, direct immersion methods are preferable whenever practicable. If the direct immersion method is not practicable an elution method can be considered. In the elution methods, an understanding of the recovery efficiency coupled with a risk assessment and rationale is critical.

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, and

— 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 ISO 11737-1:2018, B.2.2. Similarly, the considerations for selecting a suitable eluent are the same as for the bioburden determination and have been described in ISO 11737-1:2018, B.2.3 and Table B.1.

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

A.6.2 Generally, it is sufficient to perform a test of sterility on a product after its removal from its packaging system and to omit the packaging system from the test. If packaging is to be tested because it is in an integral part of the product, it should be noted that many packaging materials float on top of the culture media. This does not allow for contact with the culture media and most of the packaging material to be tested. When this is the case, attempts should be made to obtain better contact between the culture media and the packaging material [see A.6.1 a)].

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

— conducting the test in a laminar flow hood, microbiological safety cabinet, or other equipment ensuring the same particulate and microbiological level, within a microbiologically controlled room or in barrier isolation in a microbiologically controlled environment [see ISO 14644-7, ISO 14698 (all parts), and EN 12469];

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

— sterilizing all equipment, materials and items used in the test;

— introducing the test utensils, culture media and test articles into the test area aseptically;

— disinfecting the package exterior prior to introduction of the test articles into the test area;

— disinfecting the surfaces in the test area;

— minimizing the manipulations required to perform the test;

— minimizing the amount of materials in the hood;

— taking care not to disrupt the airflow patterns during manipulation;

— training in the performance of aseptic techniques.

A.6.4 To perform tests of sterility by performing an elution of product followed by directly culturing the eluate, one approach is to use culture 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-concentration culture medium in sterile containers and incubated. Alternatively, if the volume of the eluate is not more than 10 % of the volume of the culture medium, the eluate can be mixed with normal concentrated culture medium in sterile containers and incubated.

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

Surfaces that have been in contact with eluate can be rinsed with a wash/rinse solution (e.g. Fluid D), further sterile eluent or solution containing a neutralizer (see A.6.6), which is also passed through the membrane filter. Thereafter, either the culture medium is transferred aseptically to the filtration unit or the membrane filter is transferred aseptically to culture 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 and is called the method suitability test (also called bacteriostasis/fungistasis test).

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

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

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

c) reduction of the concentration of the microbicidal or microbiostatic substance to an ineffective level by dilution.

NOTE This can be achieved by increasing the volume of culture medium or eluent and, where necessary, subdividing the product into a number of test containers.

Microbicidal or microbiostatic substances can bind to filter membranes. Care should be taken to ensure the use of suitable filter membranes to minimise the potential for binding.

Guidance on the procedures, organisms, titers and incubation times for method suitability can be found in current Pharmacopeias (see [31], [32], [33], and [34]). However, the incubation temperature(s) and medium (culture media) have to be the same as those to be used in performing tests of sterility.

Multiple attempts should be made using different culturing conditions to eliminate or reduce inhibitory substances to the point where there is no longer an unacceptable risk. After multiple attempts are made, if the inhibitory substance is not eliminated, it is appropriate to accept a reduction of inhibitory substances, with an accompanying rationale and risk assessment.

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

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

The incubation temperature recommended for tests of sterility could be lower than that recommended for determination of bioburden.

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 culture medium to be used, or

— the use of a single set of culture conditions is not appropriate because of the types of microorganism likely to be present on the product and 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 could provide a rationale for the selection of culture conditions.

A.6.8 The time interval between exposure to the sterilizing agent and transfer to culture conditions should be minimized to enhance the recovery of microorganisms. Every effort should be made to carry out tests of sterility on product items or SIPs as quickly as possible after exposure to the sterilizing agent. If delay in transfer is unavoidable, the conditions under which the product items are stored should be selected to prevent loss of viability of microorganisms or changes in the microbial population.

A.6.9 Macroscopic examination is typically used to examine the medium for growth after incubation. Evidence of growth can include turbidity, pellicles, sediment, flocculation and colour change. Generally, when product items are positive for microbial growth, the microorganism(s) should be identified.

Visual examination can be undertaken with backlighting to assist in detection of turbidity.

Turbidity might not be due to the growth of microorganisms. Turbidity due to microbial growth can be verified by:

a) microscopic examination;

b) transferring portions (each not less than 1 ml) of the turbid medium to fresh containers of the same medium and incubating the subcultured containers for at least 4 days; or

c) subculturing the turbid medium using other commonly accepted microbiological practices (for example, streaking for isolation onto solid culture media).

A.7 Assessment of method for performing tests of sterility

In assessing the method for performing tests of sterility, consideration is given to the possibility of incorrect results due to false positives or false negatives.

The occurrence of false positives in tests of sterility can affect the interpretation of data obtained in validation by making a treatment with the sterilizing agent appear less effective. Unless otherwise demonstrated, positives have to be regarded as having been derived from microorganisms surviving treatment with the sterilizing agent. Factors which might affect the occurrence of false positives include:

— a breach in the sterile barrier;

— contamination during testing;

— contamination from handling during incubation.

The occurrence of false negatives in tests of sterility can affect the interpretation of data obtained in validation by making a treatment with the sterilizing agent appear more effective. Factors that might affect the occurrence of false negatives include:

— the inability of the culture conditions to support the growth of the surviving microorganisms;

— the presence of microbicidal and/or microbiostatic substances released from the product during the test of sterility (see A.6.6);

— the interval of time between treatment with the sterilizing agent and exposure to culture conditions allowing for microorganisms to lose viability (see A.6.8).

If the occurrence of positive tests of sterility can be ascribed to incorrect performance of tests of sterility, a sterilizing agent-related issue, or another relevant cause, corrective action can be implemented and a repeat test of sterility can be performed.

A.8 Maintenance of the method for performing tests of sterility

A.8.1 Because the test of sterility is vital to support definition, validation and maintenance of a sterilization process for a product or product family, a change to product, the processes used to manufacture product, sterilization process or to the parameters of the test of sterility, necessitates consideration of the need to demonstrate ongoing method suitability. Consideration should be given to the effects of cumulative changes over time. Changes to the test of sterility should be carried out within a documented change control process.

Even in the absence of planned changes to product, the processes used to manufacture product, or to the parameters of the test of sterility, consideration should be given to periodically reviewing ongoing method suitability to ensure that an accumulation of minor changes over time has not occurred that could adversely affect the continued suitability of the test method.

A.8.2 See A.8.1.