List of Titles

American National Standard

ANSI/AAMI/ISO 11737–2:1998

Sterilization of medical devices—Microbiological methods—Part 2: Tests of sterility performed in the validation of a sterilization process

AAMI Association for the Advancement of Medical Instrumentation

American National Standard ANSI/AAMI/ISO 11737-2–1998

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Approved 17 August 1998
Association for Advancement of Medical Instrumentation

Approved as an American National Standard (ANSI/AAMI/ISO 11737-2:1998) on 7 September 2000 by American National Standards Institute, Inc.

Abstract: This AAMI standard specifies the general criteria for tests of sterility on medical devices which have been exposed to a treatment with the sterilizing agent that is a fraction of the specified sterilization process.

Keywords: health care products, medical equipment, SIP, package

List of Titles

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Committee representation

Association for the Advancement of Medical Instrumentation

Sterilization Standards Committee

The adoption of this International Standard as an AAMI Standard was approved by the AAMI Microbiological Methods Working Group, under the auspices of the AAMI Sterilization Standards Committee. Committee approval of the recommended practice does not necessarily imply that all committee, sub-committee, and working group members voted for its approval.

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NOTE—Participation by federal agency representatives in the development of this standard does not constitute endorsement by the federal government or any of its agencies.

Background of AAMI adoption of ISO 11737-2:1998

Sterilization of medical devices —Microbiological methods —
Part 2: Tests of sterility performed in the validation of a sterilization process

The International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of this standard.

This part of ISO 11737 was developed by ISO Technical Committee 198 to fill a need for an international standard for tests of sterility performed in the validation of a sterilization process. ISO approved and published the standard in 1998. U.S. participation in ISO/TC 198 is organized through the U.S. Technical Advisory Group for ISO/TC 198, administered by the Association for the Advancement of Medical Instrumentation (AAMI). The United States made a considerable contribution to this standard and holds the convenership of the ISO/TC 198 working group on microbiological methods.

The AAMI Microbiological Methods Working Group of the AAMI Sterilization Standards Committee reviewed ISO 11737-2 and agreed to adopt it as a new AAMI Standard. 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 is a fraction of the specified sterilization process. This document, in conjunction with ANSI/AAMI/ISO 11737-2:1995, *Sterilization of medical devices—Microbiological methods—Part 1: Estimation of the population of microorganisms on products,* supersedes AAMI TIR 8, *Microbiological methods for gamma irradiation sterilization of medical devices.*

The concepts incorporated in this standard should not be considered inflexible or static. This standard, like any other, must be reviewed and updated periodically to assimilate progressive technological developments. To remain relevant, it must be modified as technological advances are made and as new data comes to light.

AAMI procedures require that standards be reviewed and, if necessary, revised every 5 years to reflect technological advances that may have occurred since publication. AAMI also encourages its committees to harmonize their work with international standards as much as possible. Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 3330 Washington Boulevard, Suite 400, Arlington, VA 22201.

NOTE: Beginning with the ISO foreword on page vii, this standard is identical to ISO 11737-2:1998.

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 nongovernmental, 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 3.

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.

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

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

— *Part 1: Estimation of the population of microorganisms on product*

— *Part 2: Tests of sterility performed in the validation of a sterilization process*

Annex A of this International Standard is for information only.

Introduction

A sterile product item is one which is free of viable microorganisms. The International Standards for sterilization of medical devices require, when it is necessary to supply a sterile product item, that adventitious microbiological contamination of a medical device from all sources be minimized by all practical means. Even so, product items produced under standard manufacturing conditions in accordance with the requirements for quality systems for medical devices may, prior to sterilization, have microorganisms on them, albeit in low numbers. Such product items are nonsterile. The purpose of sterilization processing is to inactivate the microbiological contaminants and thereby transform the nonsterile items into sterile ones.

The inactivation of a pure culture of microorganisms by physical and/or chemical agents used to sterilize medical devices often approximates an exponential relationship; 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 of items subjected to sterilization processing cannot be guaranteed, and the sterility of the processed population of items has to be defined in terms of the probability of the existence of a nonsterile item in that population.

Requirements for the quality system for the design/development, production, installation and servicing of medical devices are given in ISO 9001 and ISO 9002 in conjunction with ISO 13485 and ISO 13488, respectively.

The ISO 9000 series of standards designates certain processes used in manufacture as 'special' if the results cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of a special process because process efficacy cannot be verified by inspection and testing of the product. For this reason, sterilization processes have to be validated before use, the performance of the process monitored routinely and the equipment maintained.

International Standards specifying procedures for the validation and routine control of the processes used for sterilization of medical devices have been prepared (see ISO 11134, 11135 and 11137). An element of this validation may consist of exposing medical devices to the sterilizing agent when the extent of treatment has been reduced relative to that which will be used in routine 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. An example of the use of such a test is in establishing a sterilizing dose for sterilization by radiation and for demonstrating the continued validity of this sterilization dose (see ISO 11137, Annex B).

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 validation of a sterilization process

1 Scope

1.1 This part of ISO 11737 specifies the general criteria for tests of sterility on medical devices which have been exposed to a treatment with the sterilizing agent that is a fraction of the specified sterilization process. These tests are intended to be performed when validating 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) performance of a pharmacopoeial test for sterility; or

NOTE 1 The performance of a) or b) above is not a requirement of ISO 11134, 11135 or 11137.

c) culturing of biological indicators, including inoculated products.

NOTE 2 Methods of culturing biological indicators are described in ISO 11138.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 11737. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 11737 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 9001:1994, *Quality Systems—Model for quality assurance in design, development, production, installation and servicing.*

ISO 9002:1994, *Quality systems—Model for quality assurance in production, installation and servicing.*

3 Terms and definitions

For the purposes of this part of ISO 11737, the following terms and definitions apply.

3.1 aerobic organism

microorganism that utilizes oxygen as the final electron acceptor during metabolism and which will only grow in the presence of oxygen

3.2 anaerobic organism

microorganism that does not utilize oxygen as the final electron acceptor during metabolism and which will only grow in the absence of oxygen

3.3 bacteriostasis/fungistasis test

test performed with selected microorganisms to demonstrate the presence of substances that inhibit the multiplication of these microorganisms

3.4 culture conditions

stated combination of conditions, including the growth medium with the period and temperature of incubation, used to promote growth and multiplication of microorganisms

3.5 facultative organism

microorganism capable of both aerobic and anaerobic metabolism

3.6 false negative

result of a test of sterility in which a true positive is interpreted as negative

3.7 false positive

result of a test of sterility in which a true negative is interpreted as a positive

3.8 growth promotion test

test performed to demonstrate that a given medium will support microbial growth

3.9 product

generic term used to describe raw materials, intermediate products, subassemblies and finished medical devices

3.10 product unit

medical device, collection of products or components within a primary package

3.11 sample item portion (SIP)

defined portion of a product unit that is tested

3.12 test of sterility

test performed to establish the presence or absence of viable microorganisms on product units (or portions thereof) when subjected to defined culture conditions

4 General

4.1 Documentation

4.1.1 Documented instructions detailing the testing technique to be employed and the use and operation of relevant equipment shall be available. These documented instructions shall be approved and controlled as specified in ISO 9001 or ISO 9002.

4.1.2 The documented instructions required by this part of ISO 11737 shall be implemented effectively.

4.1.3 Records of original observations, derived data and final reports shall be retained in accordance with ISO 9001 or ISO 9002. The records shall include the identity of personnel involved in sampling, preparation and testing.

NOTE Software used for data capture and transfer should be validated prior to use.

4.2 Personnel

4.2.1 Responsibility for performing tests of sterility shall be assigned to specific personnel as specified in ISO 9001 or ISO 9002.

4.2.2 Training shall be performed in accordance with documented procedures, and records of the relevant qualifications, training and experience of technical personnel shall be maintained.

4.3 Equipment and materials

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

4.3.2 All equipment requiring planned maintenance shall be maintained in accordance with documented procedures. Records of maintenance shall be retained.

4.3.3 An effective system shall be established, documented and maintained for the calibration of all equipment with measurement or control functions. This calibration system shall comply with ISO 9001 or ISO 9002.

4.3.4 Methods shall be established and documented for the preparation and sterilization of materials such as glassware, growth media and eluents used in performing tests of sterility, including appropriate quality tests.

4.3.5 Quality tests for each batch of growth medium shall include a growth promotion test.

5 Selection and preparation of product units for testing

5.1 Selection

5.1.1 Product unit selection

The procedures for selection and procurement of product units for testing shall be established to ensure that these product units are representative of routine production.

5.1.2 Sample item portion (SIP)

If an SIP is used in testing, it shall be selected to possess microorganisms representative of those on the product unit.

If it has been demonstrated that the microorganisms are evenly distributed on the product unit, the SIP shall be selected from any single location on the product unit. In the absence of such a demonstration, the SIP shall be constituted from several portions of a product unit selected at random.

NOTE The standards specifying the requirement for validation and routine control of the sterilization process should stipulate the criteria for the adequacy of the SIP.

5.2 Packaging of product units and SIPs

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

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

b) the microbiological status of the product unit or the SIP is maintained;

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

6 Tests of sterility

6.1 There are two general approaches in the performance of tests of sterility. These are:

a) direct immersion of the product in growth medium or growth medium into the product, followed by incubation; and

b) removal of microorganisms from the product by elution, and transfer of the removed microorganisms to culture conditions.

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

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

b) the physical and/or chemical nature of the product to be tested;

c) possible type(s) of contaminating microorganisms and their locations on or within the product.

6.3 If microorganisms are to be removed from product before transfer to culture conditions, the factors to be considered shall also include:

a) selection of an appropriate eluent;

b) ability of the elution technique to remove contaminating microorganisms;

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

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

6.5 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 documented.

7 Assessment of method for test of sterility

7.1 The appropriateness of the methods selected for the test of sterility shall be assessed and the results of the assessment shall be documented (see 4.1.3).

NOTE 1 Actions taken under 6.4 and 6.5 should minimize the occurrence of false negatives.

NOTE 2 The correct application of the method by qualified and trained personnel should minimize the occurrence of false positives.

7.2 Modifications to the product and/or manufacturing process shall be reviewed formally to determine whether they are likely to require a change in the method for the tests of sterility. If the review indicates that a change is required, the procedures given in clause 6 shall be repeated.

Annex A
(informative)

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

A.1 Introduction

This annex provides guidance on the implementation of the specified requirements. The guidance given is not intended to be exhaustive, but to highlight important aspects that should be given attention.

This annex is not intended as a checklist for assessing compliance with the requirements.

A.2 General

A.2.1 Laboratory quality systems

In order that the data obtained from performing tests of sterility will be reliable and reproducible, it is important that the tests be performed under controlled conditions. The laboratory facilities used for the tests, whether on the site of the medical device manufacturer or at a remote location, should therefore be managed and operated in accordance with a documented quality system.

If tests of sterility are performed in a laboratory under the direct management of the medical device manufacturer, the operation of the laboratory should be within the manufacturer's quality system. If an external laboratory is used, it is recommended that such a laboratory be formally certified against an appropriate International Standard (e.g. ISO/IEC Guide 25).

Any laboratory should be committed to providing a quality service, and this commitment should be documented as a quality policy. The lines of authority and responsibility within the laboratory organization should be formally established and documented. An individual should be nominated to be responsible for the establishment of the laboratory quality system and have sufficient authority to ensure that the system is implemented.

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

Further information on quality management is available in ISO 9004. ISO/IEC Guide 25 outlines requirements for laboratory quality systems. Particular requirements for quality systems for the manufacture of medical devices are available in ISO 13485 and ISO 13488.

A.3 Apparatus and materials

A.3.1 Electronic data processing

A.3.1.1 Computers may be used in laboratories for both direct and indirect collection, processing and/or storage of data. Both the hardware and software used for such applications should be controlled.

A.3.1.2 The computer system in use should be identified, both in terms of hardware and software, and any changes in either of these aspects should be documented and subject to appropriate approval.

For software, there should be documentation describing:

a) applications software run on the computer system;

b) operations software;

c) data packages in use.

A.3.1.3 All software used in laboratories for the collection, processing and/or storage of data should be acceptance tested before being put into service (see, for example, ISO 9000-3).

A.3.1.4 If commercial software packages are purchased, these packages should have been prepared under a quality system as described in ISO 9000-3.

A.3.1.5 If computer software is developed in-house, suitable procedures should be developed to ensure that:

a) documentation on development, including the source code, is retained;

b) records of acceptance testing are retained;

c) modifications to programs are documented;

d) changes in equipment are documented and formally tested before being put into use.

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

A.3.1.6 There should be procedures to detect or prevent unauthorized software program changes.

A.3.1.7 Software programs which organize, tabulate, subject data to statistical or other mathematical procedures, or which otherwise manipulate or analyze the electronically stored data, should permit retrieval of the input data entries. Access to the source data should be available. Special procedures for archiving computer data will be required and these procedures should be documented.

A.3.2 Laboratory apparatus

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

Any apparatus, or parts thereof, that comes into contact with product, eluent, growth media, etc. during testing should be sterilized.

A.3.3 Microbiological growth media

All microbiological growth media and eluents used to remove microorganisms from product should be prepared in a manner that ensures their sterility. The sterility should be demonstrated by

a) incubation at appropriate temperatures before or concurrent with use, or

b) validation of the sterilization process.

The ability of microbiological growth medium to support growth of microorganisms should be established. This is commonly achieved by performing a growth promotion test on each batch of growth medium using an inoculum of low numbers (between 10 and 100 colony-forming units) of selected microorganisms. Growth support tests are generally described in pharmacopoeial monographs, and these monographs detail which microorganisms may be used.

A.4 Selection and preparation of product units for testing

A.4.1 Method of selecting product units for validation

The method of selecting product units for validation of a sterilization process can influence the results observed. It is preferred to select product units at random. The product units may be chosen from routine production of a batch which is representative of processing procedures and conditions; in which case, product units produced at different times during the manufacture of a single batch should be included. If a number of batches are manufactured concurrently, product units may be selected from each batch. Product units for testing may be selected from items rejected during the manufacturing process, provided that they have been subjected to the same processing and conditions as the remainder of the batch.

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

A.4.2 Sample item portion (SIP)

Whenever it is practical, an entire product unit should be used for testing, but it is recognized that this is not always possible. In such situations, a selected portion of a product unit (sample item portion, SIP), which is convenient to handle during testing, may be substituted. The SIP should be as large a portion of the product unit as is possible to manipulate readily in the laboratory.

The microbial contamination on the SIP has to represent the microbial challenge presented to the sterilization process. The SIP itself has to represent the diverse elements of the complex product units. A complex device often constitutes a greater challenge than a small device, for instance, by integrated long, narrow tubings, mated surfaces, etc. When the product is divided into different parts, this challenge often is reduced.

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.

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

considered positive. SIP can be selected on the basis of length, mass, volume, or surface area of the product unit to be tested. See table A.1 for example.

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

Table A.1—Examples for SIP selection

Basis for SIP selection	Product examples
Surface area	Implants (non-absorbable)
Mass	Powders Surgical gowns/drapes Implants (absorbable)
Length	Tubing (consistent diameter)
Volume	Water, fluids
Fluid path	Intravenous delivery set, fluid bag

A.4.3 Sample item portion for kits

A kit is considered to be a product unit containing more than one medical device; these may be a) multiple units of identical items, or b) a variety of procedure-related items.

a) **Kits containing multiples of the same medical device.** The SIP for such kits is based upon a single item and not the summation of all the items in the kit. For example, for a kit containing five syringes, one syringe is tested in its entirety and that SIP = 1.0.

b) **Kits containing different medical devices.** The SIP for such kits is based upon each type of item and a separate SIP is established for each item in the kit. For example, for a kit containing two gowns, two towels, two pairs of gloves, and a drape, an individual SIP will need to be determined for each type of item independent of the other items in the kit.

A.4.4 Packaging of product units

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

NOTE 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 may alter the chemical environment of the microorganisms.

A.5 Test of sterility

A.5.1 Categories

As indicated in clause 6 of this part of ISO 11737, the method of performing the test of sterility can be broadly divided into two general categories as follows:

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

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

Direct immersion is the preferred method of performing the test of sterility for medical devices. When it is not possible to use this technique due to characteristics of the medical device, such as bacteriostatic/fungistatic activity, the technique employing removal of microorganisms may be necessary. Care should be exercised in using this technique. An inability to remove all microorganisms from surfaces can result in the occurrence of false negatives and contamination occurring during associated manipulations can result in the occurrence of false positives.

A.5.2 Direct immersion

With direct immersion, the product unit or SIP is placed aseptically into a container (or multiple containers, see A.4.2) of growth medium and incubated. A sufficient amount of growth medium should be used to achieve contact between the growth medium and the whole of the product unit or SIP. Additionally, consideration should be given to

a) disassembly prior to exposure to the sterilizing agent (see also A.4.4);

b) agitation after placement in growth medium; or

c) the addition of a surfactant (which has been approved to have no antimicrobial effects) to the growth medium or eluent in order to improve a moistening of the product surface.

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

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

A.5.3 Removal of microorganisms

A.5.3.1 General

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

a) elution and membrane filtration;

b) elution and culturing of the eluent.

In both these subdivisions, the initial action is to remove microorganisms from the product unit or SIP. The techniques employed are the same as those used in bioburden estimation and have been described in A.4.2.4.1 to A.4.2.4.7 of ISO 11737-1:1995. Similarly, the considerations for selecting a suitable eluent are the same as for the bioburden estimation and have been described in A.4.2.5 and A.4.3 and Table A.2 of ISO 11737-1:1995.

NOTE This technique may not elute all microorganisms from the product unit.

Once the microorganisms have been removed from the product unit or SIP, the test of sterility may be performed by using membrane filtration or culture of the entire eluent.

A.5.3.2 Membrane filtration

To perform the test of sterility using membrane filtration, the eluent is passed through a sterile membrane filter, generally rated at 0.45 µm, with the aid of vacuum or pressure.

The surfaces which have been in contact with eluent are rinsed with further sterile eluent or solution containing a neutralizer, and the rinsing solution is passed through the membrane filter (see A.6.2.3). Thereafter, either

a) the growth medium is transferred aseptically to the filter; or

b) the filter is transferred aseptically to the growth medium.

Both of these operations are followed by incubation.

A.5.3.3 Culturing of eluent

To perform the test of sterility by culturing the eluent, one approach is to use growth medium as the eluent and, after elution, to transfer this growth medium to sterile containers and incubate.

Alternatively, an eluent which does not support microbial growth may be selected and, after elution, the eluent is mixed with an equal volume of double-strength growth medium in sterile containers and incubated.

A.5.4 Selection of culture conditions

A.5.4.1 The particular International Standard for validation and routine control of the sterilization process may recommend the culture conditions to be employed in the test of sterility.

A.5.4.2 Generally, a single culture growth medium may be used on the assumption that it will be optimal for the culturing of aerobic and facultative microorganisms which may survive exposure to the sterilizing agent. When using Soybean-Casein Digest Medium as a single growth medium for the culture of aerobic and facultative microor-

ganisms, culture conditions employing (30 ± 2) °C for 14 days are commonly employed. When another test growth medium is used in the test of sterility, other incubation conditions should be considered.

NOTE The incubation temperature recommended for tests of sterility may be lower than that recommended for bioburden testing. The use of the lower temperature may aid in the recovery of damaged or injured microorganisms.

A.5.4.3 A choice of culture conditions has to be made if:

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

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

A.5.4.4 Factors to be considered in choosing culture conditions in these instances should include the following:

a) the nature of the product;

b) the method of manufacture;

c) the sources of potential microbial contamination; and

d) the types of microorganisms likely to be encountered.

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

A.5.4.5 Table A.2 lists examples of commonly used culture conditions.

Table A.2—Commonly used culture conditions

Organism types	Commonly used growth media	Incubation temperature °C [a]
Aerobic	Nutrient broth	28 to 32
	Brain heart infusion broth	28 to 32
	Soybean-casein digest broth	28 to 32
Fungi	Sabouraud dextrose broth	20 to 25
	Potato dextrose broth	20 to 25
	Glucose peptone broth	20 to 25
Facultative	Fluid thioglycolate medium	28 to 32
	Cooked meat glucose broth	28 to 32
[a] Other incubation temperatures may be considered. Also see NOTE in A.5.4.2.		

A.5.5 Examination of growth medium after test of sterility

The examination of growth medium after incubation may be undertaken with backlighting to assist in detection of turbidity.

In addition, turbidity may not be due to the growth of microorganisms, therefore subculturing or microscopic examination may be required if turbidity is observed.

A.6 Assessment of tests of sterility

A.6.1 Assessment for false positives arising from contamination in performance of tests of sterility

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. Positives have to be regarded as having been derived from microorganisms surviving treatment with the sterilizing agent.

As part of the training of personnel and to assess the procedures employed in the test of sterility, a simulated test should be carried out using representative product units that have been sterilized.

Precautions that can be used to minimize the occurrence of false positives arising from contamination are listed in table A.3.

Table A.3—Precautions to minimize false positives arising from contamination

Conduct the test within the confines of a laminar flow hood located in a dedicated, environmentally controlled room.
Use aseptic techniques throughout the performance of the test (e.g. actions taken in the gowning room, during the test and during transfer of growth media to the incubators).
Introduce the test utensils, growth media and test articles into the test area in such a way so as to avoid contamination. Decontaminate the package exterior prior to introduction of the test articles into the test area.
Decontaminate test surfaces.
Sterilize all equipment, materials, and items used in the test.
Minimize the manipulations required to perform the test.
Evaluate and control the environment of the incubator.
Minimize the production of aerosols.

A.6.2 Assessment for false negatives in performance of tests of sterility

A.6.2.1 Factors which affect the occurrence of false negatives

Factors which affect the occurrence of false negatives include:

a) the inability of the culture conditions to support the growth of microorganisms;

b) the presence of microbicidal and/or microbiostatic substances released from the product during the test of sterility;

c) the interval of time between treatment with the sterilizing agent and exposure to culture conditions.

A.6.2.2 Growth-promoting qualities of medium

In selecting the growth medium, it is essential that consideration be given to its ability to support the growth of microorganisms which typically occur on or in particular product units or SIPs (see A.5.4). Once the selection has been made, the fertility of the growth medium towards typical microorganisms is established (see A.3.3).

A.6.2.3 Test for microbicidal and/or microbiostatic substances

An approach for screening for the presence of microbicidal and/or microbiostatic substances is described in ISO 11737-1:1995, annex B.4. If microbicidal or microbiostatic substances are detected, their influence may be minimized by:

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

b) removal of the microbicidal or microbiostatic substance from an eluent by filtration (see A.5.3.2);

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 or by subdividing the product units into a number of test containers (see also A.4.2).

A.6.3 Time between exposure and the test of sterility

Every effort should be made to carry out tests of sterility on product units or SIPs as quickly as possible after exposure to the sterilizing agent. If delay in transfer is unavoidable, the conditions under which the product units are stored should be selected to prevent loss of microorganisms or changes in the microbial population. The maximum interval of time before the performance of the test should be specified. Desiccation can be the cause of significant

decreases in numbers of microorganisms and should be considered in the selection of storage conditions and storage times. Furthermore, the time interval between exposure to the sterilizing agent and transfer to culture conditions may influence repair of the damage induced by the sterilizing agent. This too may have to be considered.

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