

American National Standard



ANSI/AAMI/ ISO 14161- 2009/ (R)2014

Sterilization of health care
products — Biological
indicators — Guidance
for the selection, use and
interpretation of results

Objectives and uses of AAMI standards and recommended practices

It is most important that the objectives and potential uses of an AAMI product standard or recommended practice are clearly understood. The objectives of AAMI's technical development program derive from AAMI's overall mission: the advancement of medical instrumentation. Essential to such advancement are (1) a continued increase in the safe and effective application of current technologies to patient care, and (2) the encouragement of new technologies. It is AAMI's view that standards and recommended practices can contribute significantly to the advancement of medical instrumentation, provided that they are drafted with attention to these objectives and provided that arbitrary and restrictive uses are avoided.

A voluntary *standard* for a *medical device* recommends to the manufacturer the information that should be provided with or on the product, basic safety and performance criteria that should be considered in qualifying the device for clinical use, and the measurement techniques that can be used to determine whether the device conforms with the safety and performance criteria and/or to compare the performance characteristics of different products. Some standards emphasize the information that should be provided with the device, including performance characteristics, instructions for use, warnings and precautions, and other data considered important in ensuring the safe and effective use of the device in the clinical environment. Recommending the disclosure of performance characteristics often necessitates the development of specialized test methods to facilitate uniformity in reporting; reaching consensus on these tests can represent a considerable part of committee work. When a drafting committee determines that clinical concerns warrant the establishment of *minimum* safety and performance criteria, referee tests must be provided and the reasons for establishing the criteria must be documented in the rationale.

A *recommended practice* provides guidelines for the use, care, and/or processing of a medical device or system. A recommended practice does not address device performance *per se*, but rather procedures and practices that will help ensure that a device is used safely and effectively and that its performance will be maintained.

Although a device standard is primarily directed to the manufacturer, it may also be of value to the potential purchaser or user of the device as a frame of reference for device evaluation. Similarly, even though a recommended practice is usually oriented towards healthcare professionals, it may be useful to the manufacturer in better understanding the environment in which a medical device will be used. Also, some recommended practices, while not addressing device performance criteria, provide guidelines to industrial personnel on such subjects as sterilization processing, methods of collecting data to establish safety and efficacy, human engineering, and other processing or evaluation techniques; such guidelines may be useful to health care professionals in understanding industrial practices.

In determining whether an AAMI standard or recommended practice is relevant to the specific needs of a potential user of the document, several important concepts must be recognized:

All AAMI standards and recommended practices are *voluntary* (unless, of course, they are adopted by government regulatory or procurement authorities). The application of a standard or recommended practice is solely within the discretion and professional judgment of the user of the document.

Each AAMI standard or recommended practice reflects the collective expertise of a committee of health care professionals and industrial representatives, whose work has been reviewed nationally (and sometimes internationally). As such, the consensus recommendations embodied in a standard or recommended practice are intended to respond to clinical needs and, ultimately, to help ensure patient safety. A standard or recommended practice is limited, however, in the sense that it responds generally to perceived risks and conditions that may not always be relevant to specific situations. A standard or recommended practice is an important *reference* in responsible decision-making, but it should never *replace* responsible decision-making.

Despite periodic review and revision (at least once every five years), a standard or recommended practice is necessarily a static document applied to a dynamic technology. Therefore, a standards user must carefully review the reasons why the document was initially developed and the specific rationale for each of its provisions. This review will reveal whether the document remains relevant to the specific needs of the user.

Particular care should be taken in applying a product standard to existing devices and equipment, and in applying a recommended practice to current procedures and practices. While observed or potential risks with existing equipment typically form the basis for the safety and performance criteria defined in a standard, professional judgment must be used in applying these criteria to existing equipment. No single source of information will serve to identify a particular product as "unsafe". A voluntary standard can be used as one resource, but the ultimate decision as to product safety and efficacy must take into account the specifics of its utilization and, of course, cost-benefit considerations. Similarly, a recommended practice should be analyzed in the context of the specific needs and resources of the individual institution or firm. Again, the rationale accompanying each AAMI standard and recommended practice is an excellent guide to the reasoning and data underlying its provision.

In summary, a standard or recommended practice is truly useful only when it is used in conjunction with other sources of information and policy guidance and in the context of professional experience and judgment.

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Sterilization of health care products — Biological indicators — Guidance for the selection, use and interpretation of results

Approved 3 September 2009 by
Association for the Advancement of Medical Instrumentation

Approved 30 September 2009 and reaffirmed 2 April 2014 by
American National Standards Institute, Inc.

Abstract: This American National Standard provides guidance for the selection, use, and interpretation of results from the application of biological indicators in the development, validation, and routine monitoring of sterilization processes

Keywords: bioburden, D value, incubation, inoculated carrier, PCD, SAL, survival/kill window, sterilization process validation

AAMI Standard

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Glossary of equivalent standards

International Standards adopted in the United States may include normative references to other International Standards. For each International Standard that has been adopted by AAMI (and ANSI), the table below gives the corresponding U.S. designation and level of equivalency to the International Standard. NOTE: Documents are sorted by international designation.

Other normatively referenced International Standards may be under consideration for U.S. adoption by AAMI; therefore, this list should not be considered exhaustive.

International designation	U.S. designation	Equivalency
IEC 60601-1:2005	ANSI/AAMI ES60601-1:2005	Major technical variations
IEC 60601-1-2:2007	ANSI/AAMI/IEC 60601-1-2:2007	Identical
IEC 60601-2-2:2009	ANSI/AAMI/IEC 60601-2-2:2009	Identical
IEC 60601-2-4:2002	ANSI/AAMI DF80:2003	Major technical variations
IEC 60601-2-19:2009	ANSI/AAMI/IEC 60601-2-19:2009	Identical
IEC 60601-2-20:2009	ANSI/AAMI/IEC 60601-2-20:2009	Identical
IEC 60601-2-21:2009	ANSI/AAMI/IEC 60601-2-21:2009	Identical
IEC 60601-2-24:1998	ANSI/AAMI ID26:2004/(R)2009	Major technical variations
IEC 60601-2-47:2001	ANSI/AAMI EC38:2007	Major technical variations
IEC 60601-2-50:2009	ANSI/AAMI/IEC 60601-2-50:2009	Identical
IEC 80601-2-30:2009	ANSI/AAMI/IEC 80601-2-30:2009	Identical (with inclusion)
IEC 80601-2-58:2008	ANSI/AAMI/IEC 80601-2-58:2008	Identical
IEC/TR 60878:2009	ANSI/AAMI/IEC TIR60878:2003	Identical
IEC/TR 62296:2009	ANSI/AAMI/IEC TIR62296:2009	Identical
IEC 62304:2006	ANSI/AAMI/IEC 62304:2006	Identical
IEC/TR 62348:2006	ANSI/AAMI/IEC TIR62348:2006	Identical
ISO 5840:2005	ANSI/AAMI/ISO 5840:2005	Identical
ISO 7198:1998	ANSI/AAMI/ISO 7198:1998/2001/(R)2004	Identical
ISO 7199:2009	ANSI/AAMI/ISO 7199:2009	Identical
ISO 8637:2004	ANSI/AAMI RD16:2007	Major technical variations
ISO 8638:2004	ANSI/AAMI RD17:2007	Major technical variations
ISO 10993-1:2003	ANSI/AAMI/ISO 10993-1:2003	Identical
ISO 10993-2:2006	ANSI/AAMI/ISO 10993-2:2006	Identical
ISO 10993-3:2003	ANSI/AAMI/ISO 10993-3:2003/(R)2009	Identical
ISO 10993-4:2002 and Amendment 1:2006	ANSI/AAMI/ISO 10993-4:2002/(R)2009 and Amendment 1:2006/(R)2009	Identical
ISO 10993-5:2009	ANSI/AAMI/ISO 10993-5:2009	Identical
ISO 10993-6:2007	ANSI/AAMI/ISO 10993-6:2007	Identical
ISO 10993-7:2008	ANSI/AAMI/ISO 10993-7:2008	Identical
ISO 10993-9:1999	ANSI/AAMI/ISO 10993-9:1999/(R)2005	Identical
ISO 10993-10:2002 and Amendment 1:2006	ANSI/AAMI BE78:2002/(R)2008 ANSI/AAMI BE78:2002/A1:2006/(R)2008	Minor technical variations Identical
ISO 10993-11:2006	ANSI/AAMI/ISO 10993-11:2006	Identical
ISO 10993-12:2007	ANSI/AAMI/ISO 10993-12:2007	Identical
ISO 10993-13:1998	ANSI/AAMI/ISO 10993-13:1999/(R)2004	Identical
ISO 10993-14:2001	ANSI/AAMI/ISO 10993-14:2001/(R)2006	Identical
ISO 10993-15:2000	ANSI/AAMI/ISO 10993-15:2000/(R)2006	Identical
ISO 10993-16:1997	ANSI/AAMI/ISO 10993-16:1997/(R)2009	Identical
ISO 10993-17:2002	ANSI/AAMI/ISO 10993-17:2002/(R)2008	Identical
ISO 10993-18:2005	ANSI/AAMI BE83:2006	Major technical variations
ISO/TS 10993-19:2006	ANSI/AAMI/ISO TIR10993-19:2006	Identical
ISO/TS 10993-20:2006	ANSI/AAMI/ISO TIR10993-20:2006	Identical
ISO 11135-1:2007	ANSI/AAMI/ISO 11135-1:2007	Identical
ISO/TS 11135-2:2008	ANSI/AAMI/ISO TIR11135-2:2008	Identical
ISO 11137-1:2006	ANSI/AAMI/ISO 11137-1:2006	Identical

International designation	U.S. designation	Equivalency
ISO 11137-2:2006 (2006-08-01 corrected version)	ANSI/AAMI/ISO 11137-2:2006	Identical
ISO 11137-3:2006	ANSI/AAMI/ISO 11137-3:2006	Identical
ISO 11138-1: 2006	ANSI/AAMI/ISO 11138-1:2006	Identical
ISO 11138-2: 2006	ANSI/AAMI/ISO 11138-2:2006	Identical
ISO 11138-3: 2006	ANSI/AAMI/ISO 11138-3:2006	Identical
ISO 11138-4: 2006	ANSI/AAMI/ISO 11138-4:2006	Identical
ISO 11138-5: 2006	ANSI/AAMI/ISO 11138-5:2006	Identical
ISO/TS 11139:2006	ANSI/AAMI/ISO 11139:2006	Identical
ISO 11140-1:2005	ANSI/AAMI/ISO 11140-1:2005	Identical
ISO 11140-3:2007	ANSI/AAMI/ISO 11140-3:2007	Identical
ISO 11140-4:2007	ANSI/AAMI/ISO 11140-4:2007	Identical
ISO 11140-5:2007	ANSI/AAMI/ISO 11140-5:2007	Identical
ISO 11607-1:2006	ANSI/AAMI/ISO 11607-1:2006	Identical
ISO 11607-2:2006	ANSI/AAMI/ISO 11607-2:2006	Identical
ISO 11737-1: 2006	ANSI/AAMI/ISO 11737-1:2006	Identical
ISO 11737-2:1998	ANSI/AAMI/ISO 11737-2:1998	Identical
ISO 13408-1:2008	ANSI/AAMI/ISO 13408-1:2008	Identical
ISO 13408-2:2003	ANSI/AAMI/ISO 13408-2:2003	Identical
ISO 13408-3:2006	ANSI/AAMI/ISO 13408-3:2006	Identical
ISO 13408-4:2005	ANSI/AAMI/ISO 13408-4:2005	Identical
ISO 13408-5:2006	ANSI/AAMI/ISO 13408-5:2006	Identical
ISO 13408-6:2006	ANSI/AAMI/ISO 13408-6:2006	Identical
ISO 13485:2003	ANSI/AAMI/ISO 13485:2003	Identical
ISO 14155-1:2003	ANSI/AAMI/ISO 14155-1:2003/(R)2008	Identical
ISO 14155-2:2003	ANSI/AAMI/ISO 14155-2:2003/(R)2008	Identical
ISO 14160:1998	ANSI/AAMI/ISO 14160:1998/(R)2008	Identical
ISO 14161:2009	ANSI/AAMI/ISO 14161:2009	Identical
ISO 14708-3:2008	ANSI/AAMI/ISO 14708-3:2008	Identical
ISO 14708-4:2008	ANSI/AAMI/ISO 14708-4:2008	Identical
ISO 14937:2000	ANSI/AAMI/ISO 14937:2000	Identical
ISO/TR 14969:2004	ANSI/AAMI/ISO TIR14969:2004	Identical
ISO 14971:2007	ANSI/AAMI/ISO 14971:2007	Identical
ISO 15223-1:2007 and A1:2008	ANSI/AAMI/ISO 15223-1:2007 and A1:2008	Identical
ISO 15225:2000 and A1:2004	ANSI/AAMI/ISO 15225:2000/(R)2006 and A1:2004/(R)2006	Identical
ISO 15674:2009	ANSI/AAMI/ISO 15674:2009	Identical
ISO 15675:2009	ANSI/AAMI/ISO 15675:2009	Identical
ISO 15882:2008	ANSI/AAMI/ISO 15882:2008	Identical
ISO 15883-1:2006	ANSI/AAMI ST15883-1:2009	Major technical variations
ISO/TR 16142:2006	ANSI/AAMI/ISO TIR16142:2005	Identical
ISO 17664:2004	ANSI/AAMI ST81:2004	Major technical variations
ISO 17665-1:2006	ANSI/AAMI/ISO 17665-1:2006	Identical (with inclusions)
ISO/TS 17665-2:2009	ANSI/AAMI/ISO TIR17665-2:2009	Identical
ISO 18472:2006	ANSI/AAMI/ISO 18472:2006	Identical
ISO/TS 19218:2005	ANSI/AAMI/ISO 19218:2005	Identical
ISO 22442-1:2007	ANSI/AAMI/ISO 22442-1:2007	Identical
ISO 22442-2:2007	ANSI/AAMI/ISO 22442-2:2007	Identical
ISO 22442-3:2007	ANSI/AAMI/ISO 22442-3:2007	Identical
ISO 25539-1:2003 and A1:2005	ANSI/AAMI/ISO 25539-1:2003/(R)2009 and A1:2005/(R)2009	Identical
ISO 25539-2:2008	ANSI/AAMI/ISO 25539-2:2008	Identical
ISO 81060-1:2007	ANSI/AAMI/ISO 81060-1:2007	Identical
ISO 81060-2:2009	ANSI/AAMI/ISO 81060-2:2009	Identical

Committee Representation

Association for the Advancement of Medical Instrumentation

Biological Indicators Working Group

The adoption of ISO 14161:2009 as an American National Standard was initiated by the AAMI Biological Indicators Working Group of the AAMI Sterilization Standards Committee. The AAMI Biological Indicators Working Group also functions as a U.S. Technical Advisory Group to the relevant work in the International Organization for Standardization (ISO). U.S. representatives from the AAMI Biological Indicators Working Group (U.S. Sub-TAG for ISO/TC 198/WG 4) played an active part in developing the ISO standard.

At the time this document was balloted, the **AAMI Biological Indicators Working Group** had the following members:

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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 ANSI/AAMI adoption of ISO 14161:2009

As indicated in the foreword to the main body of this document (page xi), 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, which was developed by ISO Technical Committee 198, *Sterilization of health care products*, to fill a need for guidance regarding the selection, use and interpretation of results of biological indicators.

U.S. participation in this ISO TC 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 U.S. TAG for ISO/TC 198 supports the guidance provided in this document to avoid misleading results when using biological indicators.

The U.S. adoption of ANSI/AAMI/ISO 14161:2009 was approved by the American National Standards Institute (ANSI) as a revision of ANSI/AAMI/ISO 14161:2000 on 30 September 2009. The major differences between the 2000 version and the 2009 version include harmonization with the ISO 11138:2006 series standards, an expanded section on application of biological indicator standards, and expanded annexes including calculation of z-value, D value calculation by survivor curve method, and Survival-kill response characteristics. The AAMI Biological Indicators Working Group (U.S. Sub-TAG for ISO/TC 198/WG 4, Biological indicators) participated in the development and initiated U.S. adoption of both ISO 14161:2000 and ISO 14161:2009.

AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised every five years to reflect technological advances that may have occurred since publication.

AAMI (and ANSI) have adopted other ISO standards. See the Glossary of Equivalent Standards for a list of ISO standards adopted by AAMI which gives the corresponding U.S. designation and the level of equivalency with the ISO standard.

As used within the context of this document, “shall” indicates requirements strictly to be followed to conform to the standard. “Should” indicates that among several possibilities, one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action should be avoided but is not prohibited. “May” is used to indicate that a course of action is permissible within the limits of the standard. “Can” is used as a statement of possibility and capability. Finally, “must” is used only to describe “unavoidable” situations, including those mandated by government regulation.

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 come to light.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 1110 N. Glebe Road, Suite 220, Arlington, VA 22201-4795.

NOTE—Beginning with the foreword on page xi, this American National Standard is identical to ISO 14161:2009.

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 14161 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 14161:2000), which has been technically revised.

Introduction

This International Standard provides guidance regarding the selection, use and interpretation of results of biological indicators when used to develop, validate and monitor sterilization processes. The procedures described in this International Standard are of a general nature and do not, of themselves, constitute a comprehensive development, validation or monitoring program with regard to the sterilization of health care products. The intent of this International Standard is not to mandate the use of biological indicators in a process but, if they are used, to provide guidance for their proper selection and use in order to obviate misleading results.

In this International Standard, users will find guidance on selection of the correct biological indicator for their particular sterilization process and critical parameters as well as guidance on its appropriate use.

The user should select a biological indicator that is appropriate for the particular process to be used. There is a wide variety of sterilization processes in common use, and biological indicator manufacturers are not able to foresee all possible uses of their product. Manufacturers, therefore, label biological indicators according to their intended use. It is the responsibility of the users of biological indicators to select, use, recover and interpret the results as appropriate for the particular sterilization process used.

The certified performance of a biological indicator can be adversely affected by the conditions of storage and transport prior to its use, by the use of the biological indicator or by the sterilizer process parameters. In addition, the incubation procedure used after exposure to the process, including outgrowth temperature and culture medium type, supplier and specific lot, can affect measured resistance as a function of recovery and growth. For these reasons, the recommendations of the biological indicator manufacturer for storage and use should be followed. After exposure, biological indicators should be aseptically transferred (if applicable) and incubated as specified by the biological indicator manufacturer.

It should be noted that biological indicators are not intended to indicate that the products in the load being sterilized are sterile. Biological indicators are utilized to test the effectiveness of a given sterilization process and the equipment used, by assessing microbial lethality according to the concept of sterility assurance level. Suitably trained personnel should conduct these studies.

Sterilization of health care products — Biological indicators — Guidance for the selection, use and interpretation of results

1 Scope

This International Standard provides guidance for the selection, use and interpretation of results from application of biological indicators when used in the development, validation and routine monitoring of sterilization processes. This International Standard applies to biological indicators for which International Standards exist.

NOTE 1 See, for example, the ISO 11138 series.

NOTE 2 The general information provided in this International Standard can have useful application for processes and biological indicators not currently addressed by existing International Standards, e.g., new and developing sterilization processes.

This International Standard does not consider those processes that rely solely on physical removal of microorganisms, e.g., filtration.

This International Standard is not intended to apply to combination processes using, for example, washer disinfectors or flushing and steaming of pipelines.

This International Standard is not intended to apply to liquid sterilization processes.

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 11135-1, *Sterilization of health care products — Ethylene oxide — Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices*

ISO 11138-1:2006, *Sterilization of health care products — Biological indicators — Part 1: General requirements*

ISO 11138-2, *Sterilization of health care products — Biological indicators — Part 2: Biological indicators for ethylene oxide sterilization processes*

ISO 11138-3, *Sterilization of health care products — Biological indicators — Part 3: Biological indicators for moist heat sterilization processes*

ISO 11138-4, *Sterilization of health care products — Biological indicators — Part 4: Biological indicators for dry heat sterilization processes*

ISO 11138-5, *Sterilization of health care products — Biological indicators — Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*

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

ISO 14937, *Sterilization of health care products — General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices*

ISO 17665-1, *Sterilization of health care products — Moist heat — Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*

ISO 18472:2006, *Sterilization of health care products — Biological and chemical indicators — Test equipment*

3 Terms and definitions

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

3.1

accreditation

procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks

NOTE 1 See ISO/IEC 17011^[3].

NOTE 2 Accreditation does not itself qualify the laboratory to approve any particular product. However, accreditation can be relevant to approval and certification authorities when they decide whether or not to accept data produced by a given laboratory in connection with their own activities.

3.2

aseptic technique

conditions and procedures used to exclude the introduction of microbial contamination

3.3

bioburden

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

[ISO/TS 11139, definition 2.2]

3.4

biological indicator

BI

test system containing viable microorganisms providing a defined resistance to a specified sterilization process

[ISO/TS 11139, definition 2.3]

3.5

D value

D₁₀ value

time or dose required to achieve inactivation of 90 % of a population of the test microorganism under stated conditions

[ISO/TS 11139, definition 2.11]

3.6

holding time

period for which the sterilization variable within the sterilizer and at all points within the load are continuously within the limits specified for the sterilization stage

3.7

inoculated carrier

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

NOTE 1 See ISO 11138-1.

NOTE 2 The test organism is a microorganism used for the manufacture of inoculated carriers.

3.8

installation qualification

IQ

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

[ISO/TS 11139, definition 2.22]

3.9

inoculation

addition of a defined amount of a characterized microbial entity into or on to an item

3.10

log reduction

LR

reduction in number of viable microorganisms, expressed in log units

3.11

operational qualification

OQ

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

[ISO/TS 11139, definition 2.27]

3.12

performance qualification

PQ

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

[ISO/TS 11139, definition 2.30]

3.13

process challenge device

PCD

item designed to constitute a defined resistance to a sterilization process and used to assess performance of the process

[ISO/TS 11139, definition 2.33]

3.14

process challenge location

PCL

site that simulates “worst case” conditions as they are given for sterilizing agent(s) in the goods to be sterilized

3.15

process parameter

specified value for a process variable

NOTE The specification for a sterilization process includes the process parameters and their tolerances.

[ISO/TS 11139, definition 2.34]

3.16

process variable

condition within a sterilization process, changes in which alter microbicidal effectiveness

EXAMPLE Time, temperature, pressure, concentration, humidity, wavelength.

[ISO/TS 11139, definition 2.35]

3.17

reference microorganism

microbial strain obtained from a recognized culture collection

[ISO/TS 11139, definition 2.39]

3.18

resistometer

test equipment designed to create defined reference combinations of the physical and/or chemical variables of a sterilization process

NOTE 1 Adapted from ISO 11138-1, definition 3.15 and ISO 18472:2006, definition 3.11.

NOTE 2 Also referred to as Biological Indicator Evaluator Resistometer (BIER).

3.19

spore-log-reduction

SLR

log of initial spore population, N_0 , minus the log of the final population, N_F

3.20

sterile

free from viable microorganisms

[ISO/TS 11139, definition 2.43]

3.21

sterility assurance level

SAL

probability of a single viable microorganism occurring on an item after sterilization

NOTE The term SAL takes a quantitative value, generally 10^{-6} or 10^{-3} . When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than an SAL of 10^{-3} .

[ISO/TS 11139, definition 2.46]

3.22

sterilization

validated process used to render product free from viable microorganisms

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

[ISO/TS 11139, definition 2.47]

3.23

sterilization cycle development

procedure for determination of the appropriate processing parameters that are consistent with attaining the desired specifications and label claims for a given product or group of products

3.24

sterilization cycle validation

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

3.25

supplier

organization or person that provides a product

EXAMPLE Producer, distributor, retailer or vendor of a product, or provider of a service or information.

NOTE 1 A supplier can be internal or external to the organization.

NOTE 2 In a contractual situation, a supplier is sometimes called "contractor."

[ISO 9000, definition 3.3.6]

3.26

survival-kill window

extent of exposure to a sterilization process under defined conditions where there is a transition from all biological indicators showing growth (survival time) to all biological indicators showing no growth (kill time)

[ISO 11138-1, definition 3.18]

3.27

third party

person or body that is recognized as being independent of the parties involved, as concerns the issue in question

NOTE 1 See ISO/IEC Guide 2^[1].

NOTE 2 Parties involved are usually supplier ("first party") and purchaser ("second party") interests.

3.28

user

person or body employing biological indicators for a given purpose

NOTE 1 See ISO 9000^[4].

NOTE 2 The user is the customer who is the recipient of a product provided by the supplier. In a contractual situation, the user is called "purchaser." The user could be the customer, beneficiary or purchaser. The user can be either external or internal to the organization and represents the "second party."

3.29

***z* value**

change in exposure temperature of a thermal sterilization process, which corresponds to a tenfold change in *D* value

NOTE See ISO 11138-3 and ISO 11138-4.

4 General

4.1 This International Standard provides guidance on biological indicators that can apply generally for any sterilization process, including new sterilization processes not yet covered by International Standards.

4.2 The use of biological indicators is normally documented in procedures and/or instructions.

NOTE Employing quality management systems such as ISO 13485^[7] usually satisfies this provision.

4.3 Biological indicators should always be used in combination with physical and/or chemical measurements in demonstrating the efficacy of a sterilizing process. When a physical and/or chemical variable of a sterilization process is outside its specified limits, the reason for the sterilizer's inability to achieve its process parameters should be evaluated and the problem corrected. Systems and/or procedures should be established to evaluate any deviations from the cycle process limits, and reasons for accepting any deviation should be fully documented.

4.4 A suitable biological indicator consists of carrier material and packaging and has a microbiological component that is known to be suitable for handling without special containment facilities. The growth conditions should be well documented, and the use of the indicator should be as simple and well described as possible to avoid misinterpretation by the user.

4.5 No formal approval system exists, internationally, for biological indicators that are marketed and used for stated purposes or under stated conditions. Some national regulatory authorities, however, have particular requirements for biological indicators and for the choice and use of biological indicators for the validation and control of products marketed as sterile or sterilized.

4.6 A biological indicator represents a microbiological challenge to a sterilization process and is used to verify that a sterilization process has the ability to inactivate microorganisms that have a known resistance to a referenced sterilization process. Test organisms employed in biological indicators typically have resistance to sterilization which exceeds that of common bioburden microorganisms, although some organisms can exhibit a resistance to sterilization in excess of that of the test organisms. The appropriate biological indicator provides a challenge to the sterilization process which exceeds that of the bioburden through a combination of population and resistance. If there is reason to believe that the goods to be processed could be contaminated with particularly resistant organisms, extended sterilization processing, based on the bioburden, could be required.

4.7 Biological indicators are not intended for use in any process other than that specified by the manufacturer on the product labeling. Microbial species and strains are selected as biological indicator candidates based on their known resistance to the specific method of sterilization as certified by the manufacturer. The use of an inappropriate biological indicator can give misleading results.

The user should ensure that the biological indicator has been qualified for use with the particular range of sterilization conditions that are used. This could require information in addition to that given in the labeling. When biological indicators are used outside reference conditions, the user can require information on the reaction expected from the indicator, e.g., the effect of sub-optimal moisture conditions on the biological indicators used in an ethylene oxide process. Users who employ biological indicators outside the manufacturer's labeled recommendations should thoroughly characterize the resistance of the biological

indicators to the particular sterilization process. The relationship of the response of the biological indicator to process parameters should be clearly demonstrated.

4.8 It is incumbent upon those responsible for the sterilization of product to ensure that the type of biological indicator employed to validate and/or routinely monitor a given sterilization process is appropriate for that use.

4.9 The manufacturer's recommendations for the use and storage of the biological indicators should always be followed. Failure to do so can compromise the integrity of the biological indicator. If the user removes the inoculated carrier from the biological indicator's primary packaging, changes in the resistance characteristics can occur. Guidance should be sought from the manufacturer on the extent of this change, or the user can evaluate changes in the resistance characteristics. The user should document that the performance characteristics of the inoculated carrier are appropriate for their use.

4.10 Biological indicators should not be used beyond the expiration date stated by the manufacturer.

4.11 Those who employ biological indicators for validation and/or routine monitoring of sterilization should be properly trained in their use. The time between completion of the sterilization process and the testing of the BI should be justified as described in 8.2.4. Transferal of microorganisms exposed to the sterilization process to the appropriate recovery medium should be done using aseptic technique.

4.12 The ISO 11138 series gives requirements for the information that the manufacturer should provide for biological indicators. The information may be provided on the label, as a packet insert or as a general specification accompanying the biological indicators. This series of International Standards also includes minimum requirements for resistance characteristics. Testing conditions and methods are given as reference methods.

4.13 Users of biological indicators come from a wide variety of industries, private enterprises and health care facilities. Users are not generally required to perform resistance assays on biological indicators but can have differing requirements for their quality assurance systems, which include audits of vendors and/or manufacturers (see 6.2.2).

4.14 The verification of resistance characteristics by the user is an alternative to and/or complementary to an audit, when necessary.

5 Characteristics of biological indicators

5.1 General

5.1.1 Biological indicators provide means to assess directly the microbial lethality of a sterilization process (see References [8] and [9]). When used in conjunction with physical and/or chemical process monitors, biological indicators can provide an indication of the effectiveness of a given sterilization process.

5.1.2 A sterilization process should be considered as satisfactory only when the desired physical and/or chemical parameters and microbiological results, as determined by an appropriate sterilization cycle development, validation and monitoring program have been realized. Failure to achieve the desired physical and/or chemical parameters and/or microbiological challenge forms the basis for declaring the sterilization process as nonconforming (see ISO 13485^[7] and ISO 9001^[22]).

5.1.3 Biological indicators consist of a defined population of test organisms presented in such a manner as to allow their recovery following sterilization processing. For example, test organisms employed for ethylene oxide sterilization processes can be spores of a suitable strain of *Bacillus subtilis* or *Bacillus atrophaeus*, as noted in ISO 11138-2. For steam sterilization or moist heat sterilization, the test organisms employed can be spores of a suitable strain of *Geobacillus stearothermophilus*, as noted in ISO 11138-3.

Test organisms other than bacterial spores can be used if they have been shown to provide appropriate resistance to the sterilization process.

5.1.4 The basis of all formulae used to determine biological indicator resistance characteristics such as *D* values is that the inactivation reaction follows first-order kinetics, with the requirement that the value for the coefficient of determination, r^2 , for the linearity of the survivor curve be not less than 0.8 (see Annex E and Annex F). The strain, the production method, the suspension fluid, the carrier and packaging materials all affect the resistance characteristics of the finished product (see ISO 11138-1).

5.1.5 The design and construction of a biological indicator can result in unique resistance characteristics and can vary depending on whether the biological indicator is intended for use in the development and validation of a sterilization process or for use in routine monitoring. If the design of the biological indicator for use in routine monitoring differs from that employed to validate the sterilization processes, the challenge to the process during validation should be correlated with the challenge to the process during routine monitoring.

5.1.6 The resistance characteristics of biological indicators vary according to the manufacturing methods and the testing conditions. Depending upon placement within the load and the specific lethal conditions at those discrete locations, biological indicators from the same lot may show different survival capabilities (see 7.2.3). Users of biological indicators should note that ten indicators spread throughout the load are not considered replicates due to the differences in lethality that exist throughout the chamber and load (see Note to 11.3.1).

5.2 Test organism suspension for direct inoculation of products

5.2.1 Direct inoculation of test organisms on or in product can be necessary in cycle development and other studies when the use of a biological indicator is not feasible. Direct inoculation can be appropriate for assessing factors such as product sterilizability, identification of the more difficult to sterilize locations within the device, and localized microbiological effects, e.g., moist heat versus dry heat environments.

The rationale for the selection of the “most difficult to sterilize” site(s) on a medical device or within a sterilization load should be documented based on experimental data or derived from prior knowledge of the particular sterilization methodology. In practice, the “most difficult to sterilize” site represents those locations that are most likely to provide high resistance to the sterilization process. One should refer to specific sterilization standards (e.g., ISO 17665-1 and ISO 11135-1) for guidance in determining and selecting difficult-to-sterilize locations.

5.2.2 To assess the efficacy of sterilization at a particular site or location on the product, the desired species and population of test organisms can be inoculated at those sites. The use of suspensions of test organisms to prepare inoculated carriers or inoculated products requires caution. The materials on to which test organisms are inoculated can alter the test organisms' resistance characteristics. The resistance can be higher or lower due to deposition as a monolayer or multilayer, coating effects, and/or bacteriostatic or bactericidal effects of the material. The methods employed to recover the test organisms following processing should be validated to ensure an adequate level of recovery from the product (see ISO 11737-1). Test organism recovery should be expressed in terms of percent recovery of the population of the original inoculum.

5.2.3 Direct inoculation of products or materials with a test organism suspension can cause prolonged or decreased survival of test organisms. This may affect the observed percent recovery of the original inoculum relative to what is expected under specified sterilization conditions. Inoculated products may be assayed with either survivor curve (enumeration/direct counting) or fraction-negative procedures (see Figure A.4). These assays require aseptic techniques.

5.2.4 The *D* value and, when appropriate, the *z* value, are constant values only under determined and defined conditions. The resistance characteristics of a spore suspension provided by a manufacturer or supplier of biological indicators might not correspond to the resistance characteristics for direct product

inoculation studies. The resistance characteristics should be measured for the carrier employed (solid carrier material or fluid) as well as for the specific sterilization cycle employed.

5.3 Inoculated carriers

5.3.1 Inoculated carriers consist of a defined population of test organisms inoculated on or in a suitable carrier material (see ISO 11138-1:2006, Annex B). Caution should be exercised to ensure that the integrity of the carrier material selected is sufficient to withstand sterilization processing without degradation and to minimize the loss of the inoculated test organisms during transport and handling.

5.3.2 The resistance characteristics of a test organism in suspension can be considerably changed upon deposition on or in carriers. Several factors can influence the resistance characteristics, such as the surface on to which the suspension is inoculated (e.g., solid materials, viscous products or fluids), the way the spores are dispersed and otherwise treated, the methods of drying, etc.

5.3.3 If an inoculated carrier is removed from the biological indicator primary package for cycle development, cycle validation studies, or for process challenge devices used for routine process monitoring, then it is the responsibility of the user to provide a rationale for this application. It should be recognized that the resistance of the microorganism on the inoculated carrier could differ from the labeled resistance of the packaged biological indicator.

5.3.4 The resistance characteristics of an inoculated carrier provided by the manufacturer of biological indicators might not correspond to the resistance characteristics established in direct product inoculation studies.

5.3.5 The carrier material should be evaluated by the biological indicator manufacturer or the user to establish that the sterilizing agent for which the biological indicator is intended neither retains nor releases inhibitory substances (e.g., sterilizing agent residuals) to such an extent that the recovery of low numbers is inhibited (see ISO 11138-1:2006, 5.2).

5.4 Self-contained biological indicators

Self-contained biological indicators consist of either a) or b).

- a) An ampoule containing growth medium and a carrier inoculated with test organisms contained within an outer vial so that the sterilizing agent obtains access to the inoculated carrier through a sterile barrier or a tortuous path.

After exposure to the sterilization process, the growth medium is brought into contact with the inoculated carrier by breaking the ampoule of growth medium, thereby eliminating the need to aseptically transfer the inoculated carrier to a separate vial of growth medium. The biological indicator manufacturers' recommendations should be followed for incubation of self-contained biological indicators.

NOTE Due to the low volume and the possibility of evaporation of the growth medium, prolonged post-exposure incubation might not be possible.

Chemical residuals resulting from processes such as ethylene oxide or vapor hydrogen peroxide can inhibit growth of surviving organisms. The biological indicator manufacturer's recommendations should be followed for proper handling (including aeration) of biological indicators prior to incubation (see 8.2.4).

- b) A hermetically sealed ampoule containing a suspension of test organisms in growth medium.

These are referred to as sealed-ampoule biological indicators. After exposure to the process, the sealed ampoule is incubated intact, and no aseptic transfer is required.

NOTE This type of indicator is sensitive only to exposure time and temperature and is primarily used to monitor moist heat sterilization of aqueous fluids.

Self-contained biological indicators are generally larger than biological indicators that consist only of an inoculated carrier in a primary packaging, and may not fit into locations within the device that represent the process challenge locations. Unless a biological indicator can be placed into a load without deforming it or otherwise potentially compromising its primary packaging, the biological indicator should not be used. Also, the user should be aware that the claimed resistance characteristics can be dependent on the air-removal method employed in the sterilization cycle.

5.5 Other biological indicators

Biological indicators in their simplest form consist of an inoculated carrier in primary packaging. The inoculated carrier can take a variety of forms, including paper strips, threads, metal coupons or other carriers suitable for inoculation. The primary packaging is chosen to permit the sterilizing agent to penetrate to the inoculated carrier while maintaining a sterile barrier after processing.

6 Selection of supplier

6.1 General

6.1.1 The user of biological indicators should, whenever possible, purchase to standard specifications, e.g., biological indicators manufactured according to specifications given in the ISO 11138 series, pharmacopoeial monographs or other applicable standards. The user should consider the particular sterilization process as the basis for the choice of biological indicator.

6.1.2 When the user has a process that requires performance characteristics that differ from the label claim for the biological indicator, it is the responsibility of the user to verify that the biological indicator has the performance characteristics needed.

6.1.3 The user of biological indicators should have a system in place to provide assurance that the biological indicators obtained consistently meet the specified characteristics. Such assurance may be provided by one or more of the following:

- a) information from the manufacturer covering the performance characteristics of the lot of biological indicators prepared;

NOTE Requirements for information supplied by manufacturers of biological indicators are provided by the ISO 11138 series.

- b) a statement of conformity from the manufacturer that the biological indicators meet the agreed specifications;
- c) if needed, various degrees of testing of each lot of biological indicators received by the user, to verify that the performance characteristics meet the agreed specifications.

6.1.4 When the user has established a high level of confidence in the supplier (see 6.1.3), the testing performed by the user can be minimal. At a minimum, the user should have a mechanism to ensure that a shipment of biological indicators contains all agreed-upon documentation, such as appropriate label information, packet inserts, storage and handling instructions, etc. There should be a mechanism to assure that the vendor continues to maintain the expected quality and manufacturing standards, such as

a vendor's or manufacturer's declaration of conformity to standards. If the user has not established the vendor relationship required to be assured of consistent biological indicator performance, additional testing could be necessary until an appropriate assurance can be established that the biological indicators meet the vendors' and/or manufacturers' label claim and/or user requirements.

6.1.5 Testing by the user, if deemed necessary, can consist of population assays and survival-kill resistance tests on samples from each new lot of biological indicators received (see also 8.6 and Clause 11). Provided that the manufacturer of biological indicators manufactures to detailed standard specifications, i.e. the ISO 11138 series, and the user uses the biological indicator as intended by the manufacturer, testing of the resistance characteristics by the user is considered unnecessary.

The manufacturer's label claims for resistance, such as *D* values, *z* values (if any) and survival-kill results are determined using a resistometer (see ISO 18472).

6.2 Documentation

6.2.1 General

6.2.1.1 The labeling requirements for biological indicators are given in ISO 11138-1:2006, 4.3.

6.2.1.2 The labeling includes the information presented on the primary and secondary packaging of the biological indicator as well as any included package inserts providing additional information beyond that which can be printed on the packaging. The purchaser might need or want a certificate of conformity to product standards and/or quality system standards to include in appropriate documentation files.

6.2.1.3 When a statement of performance or compliance to a standard is provided by the manufacturer as a certificate, the user should have confirmation of the competence of the manufacturer as indicated by the second sentence of 6.2.2.1.

6.2.1.4 If an independent (third-party) test laboratory is used to confirm the performance characteristics of biological indicators, the test laboratory should be accredited for the specific test methods used (see ISO/IEC 17025^[2] and ISO/IEC 17011^[3]).

6.2.1.5 The status of the manufacturer of the biological indicator with regard to conformance to the appropriate quality standard, such as ISO 13485^[7] or other quality assurance programs should be verified. If compliance to the appropriate standards can be demonstrated, an audit may not be necessary.

6.2.2 Manufacturer audit

6.2.2.1 If necessary, the user should confirm that a qualified auditing body, e.g., a certification body, has performed an audit of the biological indicator manufacturer. Alternatively, the user could perform the audit.

NOTE The auditing standard ISO 19011^[5] gives guidance on requirements for the process of auditing, the qualification criteria for quality system auditors, and the management of audit programs.

6.2.2.2 A qualified auditor should perform the audit, as part of the purchaser's quality system. If an audit of the biological indicator manufacturer is performed, the following should be considered:

a) test organism:

- 1) strain selection and maintenance;
- 2) propagation of test organisms, including growth medium and components, growth temperature and incubation period;

- 3) harvesting, purity and cleanliness of the test organism;
 - 4) viable test organism count and biochemical characteristics of the test organism;
- b) biological indicator:
- 1) qualifying of components for use in the preparation of biological indicators, such as carrier material and primary packaging, and consideration of any potential toxic effects of these materials on the test organisms;
 - 2) population of the test organisms during manufacture of the biological indicators;
 - 3) consistency (e.g., growth promotion, pH, stability, etc.) and fill volume of any growth medium supplied with the biological indicator;
 - 4) resistance of the biological indicator, including the type of test equipment and its calibration, the recovery medium employed and incubation conditions;
 - 5) storage stability and continued resistance of the biological indicator until its expiration date;
- c) quality control:
- 1) label claims for the finished product;
 - 2) storage stability and continued conformance of the finished product to its label claims.

6.2.2.3 The manufacturer should be able to provide adequate documentation of the quality systems pertaining to the manufacturing of biological indicators and provide documentation of conformity of products to declared specifications.

7 Biological indicators in process development

7.1 General

7.1.1 For additional information on process development, refer to the sterilization standards for those processes (e.g., ISO 17665-1, ISO 11135-1 and ISO 14937).

7.1.2 If a biological indicator is used for process development, the appropriateness of the indicator should be determined.

7.1.3 Sterilization processes vary widely with regard to their operational characteristics and the type of products that are sterilized. While each application is unique, it can be acceptable to group similar products in the same category for the purposes of the development, validation and routine monitoring of a sterilization process. Careful consideration should be given to those aspects of product design or packaging which can impart an additional challenge to the sterilization process. Biological indicators can be used to determine those locations on the product which represent a rigorous challenge to the process, and likewise to establish the extent to which different types of product are related with regard to the challenge presented to the sterilization process. This could lead to the selection of a particular product configuration for further analysis.

7.1.4 Physical and/or chemical parameters and microbiological results should be reviewed and interpreted for acceptability prior to acceptance of the process.

7.1.5 Biological indicators should always be used in combination with appropriate physical and/or chemical measurements of the process parameters in order to demonstrate the efficacy of a sterilization process.