List of Titles

American National Standard

ANSI/AAMI/ISO 14161:2000

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

AAMI Association for the Advancement of Medical Instrumentation

The 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 fume of reference for device evaluation. Similarly, even though a recommended practice is usually oriented towards health care 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 decisionmaking.

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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American National Standard

ANSI/AAMI/ISO 14161:2000
(Revision of ANSI/AAMI ST34:1991)

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

Approved 16 October 2000 by
Association for the Advancement of Medical Instrumentation

Approved 14 November 2000 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, incubation, inoculated carrier, PCD, PCL, process, validation

AAMI Standard

This Association for the Advancement of Medical Instrumentation (AAMI) standard implies a consensus of those substantially concerned with its scope and provisions. The existence of an AAMI standard does not in any respect preclude anyone, whether they have approved the standard or not, from manufacturing, marketing, purchasing, or using products, processes, or procedures not conforming to the standard. AAMI standards are subject to periodic review, and users are cautioned to obtain the latest editions.

CAUTION NOTICE: This AAMI standard may be revised or withdrawn at any time. AAMI procedures require that action be taken to reaffirm, revise, or withdraw this standard no later than 5 years from the date of publication. Interested parties may obtain current information on all AAMI standards by calling or writing AAMI.

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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 60601-2-21:1994 and Amendment 1:1996	ANSI/AAMI/IEC 60601-2-21 & Amendment 1:2000 (consolidated texts)	Identical
IEC 60601-2-24:1998	ANSI/AAMI ID26:1998	Major technical variations
ISO 5840:1996	ANSI/AAMI/ISO 5840:1996	Identical
ISO 7198:1998	ANSI/AAMI VP20:1994	Major technical variations
ISO 7199:1996	ANSI/AAMI/ISO 7199:1996	Identical
ISO 10993-1:1997	ANSI/AAMI/ISO 10993-1:1997	Identical
ISO 10993-2:1992	ANSI/AAMI/ISO 10993-2:1993	Identical
ISO 10993-3:1992	ANSI/AAMI/ISO 10993-3:1993	Identical
ISO 10993-4:1992	ANSI/AAMI/ISO 10993-4:1993	Identical
ISO 10993-5:1999	ANSI/AAMI/ISO 10993-5:1999	Identical
ISO 10993-6:1994	ANSI/AAMI/ISO 10993-6:1995	Identical
ISO 10993-7:1995	ANSI/AAMI/ISO 10993-7:1995	Identical
ISO 10993-8:2000	ANSI/AAMI/ISO 10993-8:2000	Identical
ISO 10993-9:1999	ANSI/AAMI/ISO 10993-9:1999	Identical
ISO 10993-10:1995	ANSI/AAMI/ISO 10993-10:1995	Identical
ISO 10993-11:1993	ANSI/AAMI 10993-11:1993	Minor technical variations
ISO 10993-12:1996	ANSI/AAMI/ISO/CEN 10993-12:1996	Identical
ISO 10993-13:1998	ANSI/AAMI/ISO 10993-13:1999	Identical
ISO 10993-15:2000	ANSI/AAMI/ISO 10993-15:2000	Identical
ISO 10993-16:1997	ANSI/AAMI/ISO 10993-16:1997	Identical
ISO 11134:1994	ANSI/AAMI/ISO 11134:1993	Identical
ISO 11135:1994	ANSI/AAMI/ISO 11135:1994	Identical
ISO 11137:1995	ANSI/AAMI/ISO 11137:1994	Identical
ISO 11138-1:1994	ANSI/AAMI ST59:1999	Major technical variations
ISO 11138-2:1994	ANSI/AAMI ST21:1999	Major technical variations
ISO 11138-3:1995	ANSI/AAMI ST19:1999	Major technical variations
ISO 11140-1:1995 and Technical Corrigendum 1:1998	ANSI/AAMI ST60:1996	Major technical variations
ISO 11607:200x [1)]	ANSI/AAMI/ISO 11607:2000	Identical
ISO 11737-1:1995	ANSI/AAMI/ISO 11737-1:1995	Identical
ISO 11737-2:1998	ANSI/AAMI/ISO 11737-2:1998	Identical
ISO TR 13409:1996	AAMI/ISO TIR 13409:1996	Identical
ISO 13485:1996	ANSI/AAMI/ISO 13485:1996	Identical
ISO 13488:1996	ANSI/AAMI/ISO 13488:1996	Identical
ISO 14155:1996	ANSI/AAMI/ISO 14155:1996	Identical
ISO 14160:1998	ANSI/AAMI/ISO 14160:1998	Identical
ISO 14161: 2000	ANSI/AAMI/ISO 14161:2000	Identical

[1)] FDIS approved; being prepared for publication.

International designation	U.S. designation	Equivalency
ISO 14937:2000	ANSI/AAMI/ISO 14937:2000	Identical
ISO 14969:1999	ANSI/AAMI/ISO 14969:1999	Identical
ISO 14937:2000	ANSI/AAMI/ISO 14937:2000	Identical
ISO 14971:2000	ANSI/AAMI/ISO 14971:2000	Identical
ISO 15223:2000	ANSI/AAMI/ISO 15223:2000	Identical
ISO 15225:2000	ANSI/AAMI/ISO 15225:2000	Identical
ISO TS 15843:2000	ANSI/AAMI/ISO TIR15843:2000	Identical
ISO TR 15844:1998	AAMI/ISO TIR15844:1998	Identical
ISO TR 16142:1999	ANSI/AAMI/ISO TIR16142:2000	Identical

Committee representation

Association for the Advancement of Medical Instrumentation

Sterilization Standards Committee

The adoption of ISO 14161:2000 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 Sterilization (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 published, the **AAMI Sterilization Standards Committee** 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 14161:2000

As indicated in the foreword to the main body of this document (page ix), 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 14161:2000 was approved by the American National Standards Institute (ANSI) as a revision of ST34:1991, *Guideline for the use of ethylene oxide and steam biological indicators in industrial sterilization processes,* on 14 November 2000. In order to preserve the useful guidance in ANSI/AAMI ST34:1991 that is not contained in ANSI/AAMI/ISO 14161:2000, the AAMI Biological Indicators Working Group has undertaken new work to develop an AAMI Technical Information Report (TIR) to retain and update that guidance. The AAMI Biological Indicators Working Group (U.S. Sub-TAG for ISO/TC 198/WG 4, *Biological indicators*) developed ANSI/AAMI ST34:1991 and initiated the U.S. adoption of ISO 14161:2000.

AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised every 5 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.

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 x, this American National Standard is identical to ISO 14161:2000.

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 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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

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

Annexes A, B, C, and D of this International Standard are for information only.

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 document 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, to avoid misleading results.

Biological indicators are not intended for use in any process other than that specified by the manufacturer on the product labeling. The use of an inappropriate biological indicator can give 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 employed. There are wide variations in sterilization processes, 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.

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, cycle parameters should be evaluated. It should be noted that measurements, which need to be evaluated, may be made during the cycle in the context of the overall cycle. 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.

The performance of a biological indicator can be adversely affected by the conditions of storage and transport prior to its use, the use of the biological indicator, the sterilizer operating parameters, or the techniques employed after exposure to the process. 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 and subjected to the validated recovery conditions as specified by the biological indicator manufacturer.

It should be noted that biological indicators are not intended to indicate that the products, nor any other load being sterilized, are sterile. Biological indicators are utilized to test the effectiveness of a given sterilization process and employed equipment 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 may 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 normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard 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 ISO and IEC maintain registers of currently valid International Standards.

ISO 11134:1994, *Sterilization of health care products—Requirements for validation and routine control—Industrial moist heat sterilization*.

ISO 11135:1994, *Medical devices—Validation and routine control of ethylene oxide sterilization*.

ISO 11138-1:1994, *Sterilization of health care products—Biological indicators—Part 1: General*.

ISO 11138-2:1994, *Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization*.

ISO 11138-3:1995, *Sterilization of health care products—Biological indicators—Part 3: Biological indicators for moist heat sterilization*.

ISO 11737-1:1995, *Sterilization of medical devices—Microbiological methods—Part 1: Estimation of population of microorganisms on product*.

ISO 13683:1997, *Sterilization of health care products—Requirements for validation and routine control of moist heat sterilization in health care facilities*.

ISO 14937, *Sterilization of health care products—General criteria for characterization of a sterilizing agent and development, validation, and routine control of a sterilization process*.

3 Terms and definitions

For the purposes of this International Standard, 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 reference [3].

NOTE 2—Accreditation does not itself qualify the laboratory to approve any particular product. However, accreditation may 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 package.

3.4 biological indicator (BI): Inoculated carrier contained within its primary pack ready for use and providing a defined resistance to the specified sterilization process.

NOTE—See ISO 11138-1.

3.5 *D*-value, D_{10} value: Time or radiation dose required to achieve inactivation of 90 % of a population of the test microorganism under stated exposure conditions.

NOTE—See ISO 11138-1.

3.6 inoculated carrier: Carrier on which a defined number of test organisms have been deposited.

NOTE 1—See ISO 11138-1.

NOTE 2—The carrier is the supporting material on which test organisms are deposited.

NOTE 3—The test organism is a microorganism used for the manufacture of inoculated carriers.

3.7 inoculation: Transferral of a defined microbial entity into or on an item.

3.8 log reduction (LR): Reduction in number of viable microorganisms, expressed in $\log_{10}$ units, after fractional exposure to a sterilization cycle.

3.9 process challenge device (PCD): Item which is deemed to present one of the greatest challenges to the effective performance of the sterilizing agent(s) in the collection of items to be sterilized.

NOTE 1—The item is so constituted that a biological indicator can be placed in the position that is most difficult for the sterilizing agent to reach.

NOTE 2—The design of the process challenge device depends on the type of goods to be sterilized and the sterilization procedure.

NOTE 3—The biological indicator should not interfere with the function of the process challenge device.

NOTE 4—In some process challenge devices an inoculated carrier may be used in place of a biological indicator.

3.10 process challenge location (PCL): Site that simulates “worst case” conditions as they are given for sterilizing agent(s) in the goods to be sterilized.

NOTE 1—The site is so constituted that a biological indicator can be placed in the position that represents a rigorous challenge for the sterilizing agent to reach.

NOTE 2—The site depends on the type of goods to be sterilized and the sterilization process parameters.

NOTE 3—The biological indicator should not interfere with the function of the goods.

NOTE 4—In some sites an inoculated carrier may be used in place of a biological indicator.

3.11 process parameter: Specified value for a process variable.

NOTE—Specifications for a sterilization process include the process parameters and their tolerances.

3.12 resistometer: Equipment designed to create defined combinations of the physical and/or chemical variables of a sterilization process within defined limits.

NOTE 1—See ISO 11138-1.

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

3.13 sterilization cycle development: Procedure for determination of the appropriate processing parameters and conditions which are consistent with attaining the desired specifications and label claims for a given product or group of products.

3.14 sterilization cycle validation: Documented procedure for obtaining, recording, and interpreting the results required to establish that a process would consistently yield product complying with predetermined specifications.

3.15 sterile: Free from viable microorganisms.

3.16 sterilization: Validated process used to render a product free from viable microorganisms.

NOTE—In a sterilization process, the nature of microbial inactivation is described by an exponential function. Therefore, the presence of viable microorganisms on any individual item can be expressed in terms of probability. While this probability may be reduced to a very low number, it can never be reduced to zero.

3.17 supplier: Organization that provides a product to the customer.

NOTE 1—In a contractual situation, the manufacturer may be called the "contractor."

NOTE 2—The supplier may be, for example, the manufacturer, distributor, vendor, importer, assembler, or service organization. The supplier can be either external or internal to the organization. The supplier is a person or business concern that manufactures goods or owns a factory and represents the "first party" (see reference [4]).

3.18 third party: Person or body that is recognized as being independent of the parties involved, as concerns the issue in question.

NOTE 1—See reference [1].

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

3.19 user: Person or body employing biological indicators for a given purpose.

NOTE 1—See reference [4].

NOTE 2—The user is the customer who is the recipient of a product provided by the supplier (see reference [4]). In a contractual situation, the user is called "purchaser." The user may be the customer, beneficiary, or purchaser. The user can be either external or internal to the organization and represents the "second party."

3.20 *z*-value: <thermal sterilization process> the change in exposure temperature which corresponds to a 10-fold change in *D*-value.

NOTE—See ISO 11138-3.

4 General

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

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

NOTE—Employing quality systems complying with ISO 13485 or ISO 13488 satisfies this provision (see references [11] and [12]).

Biological indicators that are defined in ISO 11138-1, ISO 11138-2, and ISO 11138-3 give requirements for the manufacture of biological indicator systems where the biological component is a microorganism, such as a bacterial endospore or other microbiological form. The ISO 11138 series gives requirements for biological indicators for use in sterilization processes. These International Standards require that suitably trained personnel carry out the procedures and methods described.

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 to the user as possible to avoid misinterpretation.

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.

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 may exhibit a resistance to sterilization in excess of that of the test organisms. The appropriate biological indicator has a combination of population and resistance that exceeds that of the bioburden. If there is reason to believe that the goods to be processed may be contaminated with particularly resistant organisms, extended sterilization processing, based on the bioburden, may be required.

The user should ensure that the biological indicator has been validated for use with the particular range of sterilization conditions that are used. This may require additional information than that given in the labeling. When biological indicators are used outside reference conditions, the user may require information on the reaction to be 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 for non-standard sterilization techniques should thoroughly characterize the resistance of the biological indicators to the particular sterilization process as compared to a wide range of microorganisms, including any hazardous microorganisms or infectious agents that may constitute a part of the bioburden of the product. The relationship of the response of the biological indicator to process parameters should be clearly demonstrated.

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.

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

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

Those who employ biological indicators for validation and/or routine monitoring of sterilization should be properly trained in their use. Post-sterilization handling of inoculated carriers and inoculated products should be performed according to validated guidelines or in compliance with the directions provided by the manufacturer of the inoculated carriers. The transfer of microorganisms exposed to the sterilization process to the appropriate recovery medium should employ aseptic technique.

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. These International Standards also include minimum requirements for resistance characteristics. Testing conditions and methods are given as reference methods.

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

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

Biological indicators provide means to assess directly the microbial lethality of a sterilization process (see references [14] and [15]). 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.

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 reference [29]).

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*, 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 *Bacillus stearothermophilus*, as noted in ISO 11138-3.

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 correlation coefficient for the linearity of the survivor curve be not less than 0.8 (see ISO 11138-1). 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).

The design and construction of a biological indicator may result in unique resistance characteristics and may 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 process, the challenge to the process during validation should be capable of correlation with the challenge to the process during routine monitoring (see annex A, Figure A.2).

5.2 Test organism suspension for direct inoculation of products

Direct inoculation of test organisms on or in product may be necessary in cycle development and other studies when the use of a biological indicator is not feasible. Direct inoculation may 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 (see annex D).

NOTE—The “most difficult to sterilize” site on a device or within a sterilization load is determined based on experimental and reproducible data derived from 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 11134 and ISO 11135) for guidance in estimating difficult-to-sterilize locations.

To assess the efficacy of sterilization at a particular site or location on the product, the desired species and population of test organisms may be inoculated at those sites that represent a rigorous challenge to the sterilization process. The use of suspensions of test organisms to prepare inoculated carriers or inoculated products requires caution. Inoculation of test organisms onto different materials may alter the resistance characteristics, causing the resistance to be higher or lower due to adhesion of spores to the material as monolayer and/or multilayer, to coating effects, to bacteriostatic or bactericidal effects, etc. Likewise, caution should be exercised with regard to the techniques employed to recover the test organisms following processing in order to ensure an adequate level of recovery from the product (see ISO 11737-1). Methods used for recovery of test organisms should be validated and expressed in terms of percent recovery of the original inoculum (see reference [24]).

For products or materials, the use of direct inoculation with a spore suspension may cause prolonged or decreased survival of spores, in terms of percent recovery of the original inoculum under normal sterilization conditions. Inoculated products may be assayed with either survivor curve (enumeration/direct counting) or fraction-negative analysis (Most Probable Number procedures) (see annex A, Figure A.4). This requires aseptic techniques.

The *D*-value and, when appropriate, the *z*-value, are only constant values under determined and defined conditions. The resistance characteristics of a spore suspension provided by a manufacturer or supplier of biological indicators may not correspond to the resistance characteristics for direct product inoculation studies. The resistance characteristics should be validated for the carrier employed (solid carrier material or fluid) as well as for the specific sterilization cycle employed.

5.3 Inoculated carriers

Inoculated carriers consist of a defined population of test organisms inoculated on or in a suitable carrier material. 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.

The resistance characteristics of a test organism in suspension may be considerably changed upon deposition on or in carriers. Several factors may influence the resistance characteristics, such as the surface onto 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.

If an inoculated carrier is removed from the biological indicator primary package to be used for cycle development or cycle validation studies or for process challenge devices for routine process monitoring, it is the responsibility of the user to validate this application. It should be recognized that the resistance of the inoculated carrier (e.g., “naked” carrier) may differ from the resistance of the biological indicator system as labeled, due to hindrance to sterilizing agent penetration by the primary packaging.

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.

The carrier material should be evaluated by the biological indicator manufacturer with the sterilizing agent for which the biological indicator is intended, to show that it neither retains nor releases inhibitory substances (e.g., sterilizing agent residuals) to such an extent that the recovery of low numbers of test organisms is inhibited subsequent to processing (see ISO 11138-1 for carrier-material validation).

5.4 Biological indicators

5.4.1 General

The resistance characteristics of biological indicators vary according to the manufacturing methods and the testing conditions. The same lot of biological indicators may also show varying resistance characteristics according to the process and placement within the load in which they are used. The user needs to document the placement of the chosen biological indicators in the sterilizer chamber location, within the product load or a process challenge device (see annex B).

5.4.2 Self-contained biological indicators

Self-contained biological indicators consist either of:

a) an ampule of 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 via a tortuous path or filter. After exposure to the sterilization process, the growth medium is brought into contact with the inoculated carrier by breaking the ampule of growth medium, thereby eliminating the need to aseptically transfer the inoculated carrier to a separate vial of growth medium; or

b) a hermetically sealed ampule containing a suspension of test organisms in growth medium. These are referred to as sealed-ampule biological indicators. After exposure to the process, the sealed ampoule is incubated intact, and no aseptic transfer is required.

NOTE 1—This type of indicator is sensitive only to exposure temperature and is primarily used to monitor moist heat sterilization of aqueous fluids.

Self-contained biological indicators are of large size compared to those biological indicators which consist only of an inoculated carrier in a primary packaging. It may not be possible to place them in locations within the device that represent the process challenge locations. Also, the user should be aware that the claimed resistance characteristics may be dependent on the air-removal method employed in the sterilization cycle.

NOTE 2—Due to the low volume and the possibility of evaporation of the growth medium, prolonged post-exposure incubation may not be possible.

5.4.3 Other biological indicators

Biological indicators which consist of an inoculated carrier within the primary package should have the inoculated carrier removed from the primary pack prior to incubation. Aseptic technique should be used when transferring the inoculated carrier to the growth medium in order to avoid contamination.

In some cases, an inoculated carrier may be removed from the primary package to be used for cycle development or cycle validation studies or for process challenge devices for routine process monitoring (see 5.3). It should be recognized that the resistance of the inoculated carrier (e.g., “naked” carrier) may differ from the resistance of the biological indicator system as labeled, due to hindrance to sterilant penetration by the primary packaging.

6 Selection of supplier

6.1 General

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.

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.

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.

When the user has established a high level of confidence in the supplier [see a), b), and c) above], the testing performed by the user may be minimal. At a minimum, the user should have a mechanism to assure that a shipment of biological indicators meets criteria established in the purchasing agreement and 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 by 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 may be necessary until an appropriate assurance can be established that the biological indicators meet the vendor label claim and/or user requirements.

Testing by the user, if deemed necessary, may 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.

6.2 Documentation

6.2.1 General

The labeling requirements for biological indicators are given in ISO 11138-1, ISO 11138-2, and ISO 11138-3.

The user may need the information on the performance characteristics of the biological indicator presented in different ways.

The information provided by the manufacturer of the biological indicator could be presented as part of the labeling. The purchaser may need or want a certificate of conformity to product standards and/or quality system standards to include in appropriate documentation files.

When assurance is provided by the manufacturer as a certificate, the user should have confirmation of the competence of the manufacturer, e.g., by third-party audit of their operation.

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 references [2] and [3]).

The status of the manufacturer of the biological indicator with regard to conformance to the appropriate ISO 9000 standard 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

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 standards ISO 10011-1, ISO 10011-2, and ISO 10011-3 give guidance on requirements for the process of auditing, the qualification criteria for quality system auditors, and the management of audit programs (see references [5], [6], and [7]).

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 of test organisms, purity and cleanliness;

4) viable test organism count and biochemical characteristics of the test organism;

5) resistance of the test organisms, including the type of test equipment and its calibration, the recovery medium employed, and incubation conditions;

6) storage stability and continued resistance of the test organism until its expiration date.

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; and

3) consistency (e.g., growth promotion, pH, stability, etc.) and fill volume of any growth medium supplied with the biological indicator.

c) quality control:

1) label claims for the finished product; and

2) storage stability and continued conformance of the finished product to its label claims.

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

For additional information on process development, refer to the sterilization standards for those processes (e.g., ISO 11134, ISO 11135, ISO 13683, and ISO 14937).

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

Sterilization processes vary widely with regard to their operational characteristics and the type of products that are sterilized. While each application is unique, it may be acceptable to group similar products in the same category for the purposes of the development, routine monitoring, and validation of a sterilization process. Careful consideration shall be given to those aspects of product design or packaging which may impart an additional challenge to the sterilization process. Through the use of biological indicators, it may be possible 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 may lead to the selection of a particular product configuration for further analysis.

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

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

General advice on the number of biological indicators per sterilizer volume cannot be given, as this depends on the reproducibility of the cycles as well as the potential for differences in the process parameters throughout the load during sterilization. The proper number of biological indicators to use should be chosen from data collected from the use of biological indicators and/or bioburden studies, as well as from documentation on the distribution of the sterilizing agent throughout the load. Generally, the greater the variability of process parameters (e.g., temperature, relative humidity, gas distribution, etc.), the more biological indicators may be required to reliably monitor the sterilization load.

Advice as to a minimum number of biological indicators for ethylene oxide processes can be found in ISO 11135.

7.2 Reference microorganism method

This method is often referred to as the “half-cycle method,” “fractional cycle method,” or “overkill sterilization method” and is discussed in ISO 14937. This method is based on the following assumption:

a) that the biological indicator provides a greater challenge than the bioburden, i.e., the reference microorganism;

b) that the full sterilization process achieves at least a twelve-log reduction of the biological indicator with minimum resistance characteristics (see annex A, Figure A.1);

NOTE 1—The minimum requirements for biological indicators are currently to be found in ISO 11138-1, ISO 11138-2, and ISO 11138-3 for ethylene oxide and moist heat (see annex A, Figure A.2).

c) that, at half cycle, the user can typically demonstrate at least a six-log reduction (see annex A, Figure A.1).

NOTE 2—ISO 11138-1, ISO 11138-2, and ISO 11138-3 permit resistance characteristics other than the minimum required for monitoring purposes (see annex A, Figure A.2).

These criteria may be addressed by placing biological indicators or inoculated carriers with, for example, a population of 10^6 test organisms meeting minimum resistance requirements at the process challenge location(s) within product throughout the load. These locations within the load should have been previously demonstrated to present a challenge to the sterilization process and correlate with the "most difficult to sterilize" locations, such that choosing these locations will secure the appropriate log reduction for the entire product load. At least a six-logarithm reduction in the population of organisms should be demonstrated within one-half the normal holding time of the cycle to be validated. The percentage of biological indicators showing growth may vary depending upon the log reduction achieved. If testing under half-cycle conditions demonstrates a reduction in the population of test organisms in excess of six logarithms, it is possible that no growth of the test organism may result, depending on the sample size. As illustrated in Figure A.1, there is a 1 % probability of positive growth with a spore log reduction of eight logarithms at the upper end of the half-cycle window (see annex A, Figure A.1).

Placement of the biological indicator either within the product or within the load is likely to alter its apparent resistance characteristics in comparison to the resistance noted on the labeling of the biological indicator. This may require adjusting the half-cycle exposure time to compensate for the additional resistance caused by the placement of the biological indicator in the product or load. Similar adjustments may be needed when test organism suspensions are used to prepare inoculated product (see 5.2).

Suitable physical and/or chemical probes should be used to establish temperature distributions, etc., that may aid in determining placement locations for the biological indicators. A sufficient number of probes should be prepared with biological indicators placed at the previously determined location within the product.

NOTE—For moist heat sterilization, the *z*-value of the biological indicator may be different from the *z*-value of 10 °C, which is normally assumed for process lethality based on temperature measurements. This may lead to discrepancies between the integrated process lethality determined by use of biological indicators and the lethality determined by direct temperature measurements.

7.3 Combined biological indicator and bioburden method

The combined biological indicator and bioburden method requires the population and microorganism resistance of the product bioburden to be known. This method has the advantage of permitting a reduction in cycle exposure time and minimizing exposure of the product to the sterilizing agent and is discussed in ISO 14937.

The combined biological indicator/bioburden methodology requires selecting process conditions which deliver a process lethality sufficient to inactivate the bioburden to the labeled product sterility assurance level. The number of replicate cycles required to demonstrate the appropriate efficacy will depend on the confidence in accuracy and degree of bioburden inactivation repeatability determined in bioburden evaluations. Annex A, Figure A.1, shows the general relationship between the inactivation of a biological indicator and the inactivation of product bioburden giving a product sterility assurance level lower than the biological indicator sterility assurance level.

For this procedure, the appropriate biological indicator with a population of test organisms per carrier of less than 10^6 may be employed. For example, the population of the biological indicator may be equal to the average bioburden population plus three standard deviations. The population of the biological indicator should not be less than 10^3 test organisms per carrier (see annex A, Figure A.1).

Procedures for the estimation of the product bioburden are discussed in ISO 11737-1. Due to bioburden variation, it may be necessary to characterize bioburden and bioburden resistance on a routine basis.

NOTE—Studies of the resistance of the total bioburden population may be required in order to assure that the challenge provided is less than that of the biological indicator. One possible method to determine this would be to run fractional sterilization cycles to determine that the bioburden population does not survive as great an exposure as does the biological indicator.

Implementation of the combined biological indicator/bioburden method requires consideration of many of the same factors noted in 5.4 and 7.2, with regard to the placement of the biological indicator in those locations within the product and load which present a rigorous challenge to the sterilization process. The method is only applicable when

data are sufficient to be subjected to valid statistical analysis and there is a high level of confidence that the bioburden data is representative of "worst-case" conditions. There are many causes of variation, such as raw materials, process control, and seasonal variations. Consideration should be given to the presence and nature of the bioburden distribution in the product. As the distribution of bioburden within the product may vary significantly, it is important to determine how this distribution may affect the challenge presented by the product to the sterilization process and thereby affect the choice of biological indicator.

The selection of a suitable method or methods to determine product bioburden and product bioburden distribution requires consideration of many factors. Regardless of which method is selected, the method employed shall be validated, and the degree of confidence in the bioburden estimate established (see the ISO 11737 series for further details regarding the selection and validation of methods for sampling product bioburden).

The fundamental criterion of the combined biological indicator/bioburden methodology requires selecting processing conditions which reduce the population of the bioburden to 10^0 with an additional safety factor consistent with the labeling of the product (see annex A, Figure A.1). The number of replicate cycles for which this should be demonstrated during sterilization cycle development depends on the accuracy and degree of confidence of the bioburden estimates.

Other strains may be employed as biological indicators even though their resistance characteristics are different from those noted in ISO 11138-2 or ISO 11138-3, as long as those resistance characteristics are demonstrated to be correlatable and higher than the overall resistance characteristics of the product bioburden.

In some cases, where the resistance characteristics of the bioburden are higher than those of commercially available biological indicators, the particular strains isolated from the bioburden should be considered for inclusion in the process development studies (see ISO 11135 and ISO 11134), or a correction should be made based on their resistance characteristics. Alternatively, a biological indicator with a higher population may be acceptable when the population and the resistance are considered together.

7.4 Bioburden method

The reader should refer to the ISO 11737 series for the appropriate microbiological methods to use to establish an estimate of the bioburden. Some bioburden microorganisms may have greater resistance than the biological indicators described in ISO 11138-1, ISO 11138-2, and ISO 11138-3. The bioburden microorganism with the higher resistance may be used as a model biological indicator (see 7.3); the method is discussed in ISO 14937.

For information on validation and routine control and the applicability of absolute bioburden methods, the reader is advised to seek information in the relevant International Standards for their particular sterilization process (see clause 2). An absolute bioburden method, without any reference to biological indicators, is given as detailed specifications in ISO 11137 and ISO/TR 13409 (see references [8] and [10]).

8 Biological indicators in sterilization validation

8.1 General

If biological indicators are used in the validation process, one should also consider the type(s) of biological indicators which may be used in the routine monitoring. Different biological indicators may provide varying degrees of challenge to the sterilization process (see Figure A.2). If different biological indicators are used for validation and routine monitoring, both should be included in the validation studies so that the resistance relationship of the two can be established and documented.

8.2 Placement and handling of biological indicators

Validation of a sterilization process requires documentation that the process is capable of consistently producing product that meets its predetermined specifications (see ISO 11134, ISO 11135, and ISO 14937).

The number of biological indicators in products and/or product loads should be documented.

Other considerations to be addressed in biological indicator placement within the product load are loading patterns, load density and geometry, process challenge location(s), placement of physical and/or chemical sensors or probes, potential stratification of physical elements, the effect of packaging, etc.

The biological indicators should be removed from the sterilizer load as soon as possible after the process without compromising the safety of personnel. They should be tested within a specified time interval that has been established and validated for that product and process. The time limit given in the ISO 11138 series of 2 h between the end of processing and the start of culturing is a specific requirement for *D*-value determination carried out by manufacturers of biological indicators. The time interval between preparation of the indicators and the sterilization process, and the time interval between the end of the process and the culturing of the indicators should be validated.

If the biological indicators are handled in a manner other than those stated by the manufacturer, the procedures should be validated to determine if they affect the performance of the biological indicator. Any validated time intervals shall be followed.

NOTE—National or regional requirements for worker safety should be observed when removing the biological indicators from the sterilizer.

8.3 Sterilizer qualification

Initial qualification of a newly installed sterilizer could be called “commissioning.” “Installation qualification (IQ)” and “operational qualification (OQ)” are terms used in ISO 14937. Initial qualification is performed to obtain and document evidence that the sterilizer, its services, and ancillary equipment have been provided and installed in accordance with its specifications, and that the sterilizer functions within predetermined limits when operated in accordance with instructions (see ISO 11134, ISO 11135, and ISO 13683).

NOTE 1—“Commissioning” may be a combination of IQ and OQ.

NOTE 2—Biological indicators may be used in OQ/PQ, for example, to establish evidence of uniformity of distribution of sterilizing agents.

Manufacturers of sterilizers may have tests performed in their factories prior to delivery using biological indicators for specific types of load (see annex B).

8.4 Performance qualification

Following completion of sterilizer qualification (see 8.3), performance qualification (PQ or process qualification) testing is conducted to document the reproducibility and the efficacy of the sterilization process, including its ability to produce product meeting its predetermined quality specifications. Relevant International Standards such as ISO 11134, ISO 11135, ISO 13683, and ISO 14937 apply. Different biological indicators may provide varying degrees of challenge to the sterilization process (see Figure A.2). Correlation between the biological indicators used for cycle development and validation, performance qualification, and routine monitoring should be established and documented.

8.5 Review and approval of validation

Upon successful completion of qualifications, a review of the validation documentation, including biological indicator performance, is necessary prior to beginning manufacture to certify that the process conforms to requirements. When using biological indicators with moist heat processes, ISO 11134:1994 subclause 7.5 applies; when using ethylene oxide sterilization, ISO 11135:1994 subclause 5.5 applies.

8.6 Requalifications

When performing requalifications, the same resistance characteristics, number of biological indicators, their placement in product load, etc., should be used. If a new biological indicator is being qualified for the process, it is important to establish and document a correlation between the new biological indicator and the previous biological indicator.

When using moist heat or ethylene oxide sterilization, ISO 11134:1994 subclause 7.6 or ISO 11135:1994 subclause 5.6 applies.

A minimum frequency for showing that the biological indicator system remains within control should be established. Considerations leading to different intervals for requalification of the biological indicator system could include seasonal changes, product and material changes as well as equipment changes, etc. If the resistance characteristics of the biological indicator change outside the predetermined limits, requalification should be performed. If the recovery medium is changed, the new growth medium should be correlated to the previous one used and the choice of new growth medium should be validated (see also 12.4).

9 Biological indicators in routine monitoring

9.1 General

Biological indicators may not be required and may provide little value in routine monitoring of some sterilization processes (e.g., moist heat sterilization, see ISO 11134 and ISO 13683). For processes where parametric release is not achievable, biological indicators provide the best available alternative for demonstrating microbial lethality in the sterilization process.

The type of biological indicator and its placement in the product or product load should be consistent with product load locations that have been determined during the sterilization development or the validation. If the microbiological

challenge system used for routine monitoring of the sterilization process differs from that used in the validation of the process, the relationship between the system for validation and for routine monitoring should be documented.

The time interval specified between preparation of the indicators and sterilization, and between sterilization and culturing the indicators, should be validated to show no adverse effects on the biological indicator performance.

For routine monitoring, the time intervals should be the same as stated for the validation process (see 8.1 and 8.2). Biological indicators for routine monitoring that are based on the biological indicator/bioburden method (7.3) may not necessarily comply with all parts of the 11138 series.

NOTE—National or regional requirements for worker safety should be observed when removing the biological indicators from the sterilizer.

9.2 Placement and handling of biological indicators

During cycle development and validation, biological indicators are placed in those sites within the product and load that present a rigorous challenge to the sterilization process. During routine monitoring, it may be desirable to place the biological indicators in more accessible locations using a process challenge device (see 9.3). In these situations the placement of the biological indicators should be correlated with the locations employed during cycle development to ensure that the integrity of the sterilization process is not compromised. Consistent placement of the biological indicators employed for routine monitoring should also be ensured.

The directions of the supplier of the biological indicator should be followed with regard to the proper handling of the biological indicator subsequent to sterilization. In general, biological indicators should be removed from the load without compromising the safety of personnel and within a specified time period that is validated (see 9.1, NOTE). They should then be aseptically transferred to the appropriate growth medium within the defined time period and incubated at the proper temperatures (see 8.2, 9.1, and clause 12).

In addition to the qualification requirements on medium growth properties (see clause 12) and viability of the biological indicator (see clause 11), the user could also perform abbreviated versions of these checks during routine monitoring of the sterilization process. For example, using an unprocessed biological indicator incubated in the growth medium indicates both the viability of the indicator and the suitability of the growth conditions.

The process should be considered acceptable only when the desired physical and/or chemical parameters have been reviewed and the microbiological results interpreted and both found to comply with the desired criteria.

9.3 Process challenge device (PCD)

A process challenge device in combination with biological indicators is used both for validation and routine monitoring of sterilization cycles as well as for sterilizer testing by sterilizer manufacturers. Process challenge devices are designed so that the placement of the biological indicator within the process challenge device constitutes a location that is deemed to represent a suitably stringent challenge to the process. The design of the process challenge device may vary according to the nature of the product to be challenged (see annex B for various examples of process challenge devices).

Process challenge devices shall be designed with consideration given to the various process parameters that influence the sterilization process. Composition of a process challenge device depends on the type of cycle to be monitored, as well as the type of product to be sterilized.

Process challenge devices can be commercially available as prefabricated sets, often called “biological test packs.” Single-use biological test packs are manufactured by various companies and may be used instead of in-house process challenge devices. Process challenge devices and their placement in the product load should represent a challenge to the process that is equivalent to or greater than the challenge represented by the product load.

10 Results

10.1 General

The criteria for acceptance of a sterilization process as satisfactory should be decided upon during the sterilization cycle development, using relevant standards for the validation and control of the sterilization process.

In order to obtain reliable results, routine procedures should be established and maintained, carried out by trained technicians using appropriate equipment.

10.2 Interpretation of results

A validated sterilization process in which all the pre-set parameters have been met should show no growth of the biological indicator.

Based on the principles of the use of biological indicators in ISO 11135, a sterilization process where pre-set minimum parameters have not been met could show growth of the biological indicator.

A sterilization process where only some of the process parameters have been met might or might not show growth of the indicator.

Actions to be taken upon growth of a biological indicator subsequent to sterilization processing may vary with institutional and regulatory policies and may require that the lot of product be rejected as non-sterile. The identification of growth as that of the test organism should be confirmed, and an effort made to identify the cause of the growth. Consistent growth of biological indicators subsequent to sterilization processing may indicate a loss of integrity of the sterilization process or an unusually high resistance of the biological indicator lot under use. If an investigation of the biological indicator indicates no significant change in the biological indicator that affects its performance in the sterilization process, then the sterilization process should be requalified (see 8.6).

The particular sterilizer, type of product, and the loading of the product all affect the sterilization process. The resistance characteristics of the biological indicator system used in the process should be established for the overall system to be effective. Acceptable data from the biological indicator are only a part of the data necessary to show that the sterilization process has been successful.

Growth of a biological indicator following a complete sterilization cycle may be an indication that the pre-set minimum parameters have not been met. A biological indicator that shows growth after sterilization is only indicative of a sterilization process failure if the resistance characteristics of the biological indicator system are suited to the sterilization system.

Any biological indicator test results showing growth of the indicator when no growth would be expected may be an indication of an invalid process, a defective biological indicator or a faulty test system and should lead to an investigation. Unsuitable systems shall be requalified. Identity of the indicator organism should be confirmed for positive biological indicators. If the identity is confirmed, the result should be interpreted as an incomplete sterilization of the product lot, unless investigation of the biological indicator failure can prove otherwise (see clause 11).

Cultures showing growth which is not confirmed to be the indicator organism should be further investigated to determine the cause of the positive. Frequent test contaminants may indicate a faulty test system or inadequate training of personnel.

Biological indicators showing growth where all the documentation shows conformance to the requirements of the process may be caused by contamination during the transfer of the biological indicator to the growth medium.

Excessive sterilization of the culture medium, insufficient availability of oxygen in the culture container, or other improper culturing conditions, depending on the culture conditions for the specific strain in use, may compromise the biological indicator system, resulting in no growth when growth should occur. Systems should be established to detect any untoward deviations. This can be achieved by establishing routines for growth promotion tests of the medium or introducing positive controls and tests as well as, for example, establishing routines for controlling the temperature of the incubator (see clause 12).

11 Application of biological indicator standards

11.1 General assessment of biological indicator performance by the user

The two main characteristics of a biological indicator are the nominal population of microorganisms and the resistance of the biological indicator to the sterilization process, expressed as the *D*-value.

The biological indicator should be transported, stored, and handled to ensure that the nominal population and resistance characteristics are maintained during the shelf life. Sterilization of culture medium, incubation conditions, equipment maintenance, and training of laboratory personnel are some areas that should be defined and controlled to ensure appropriate performance of the biological indicator. The user may periodically verify the biological indicator population. When the above-mentioned areas are controlled and validated, routine biological indicator testing by the user may not be necessary.

The user should note any deviation from the process that is employed and the reference set of parameters that has been defined for the process. If the sterilizer cycle parameters or load are the reason for the deviations in the biological indicator's resistance characteristics, the user should investigate the possibility of eliminating these variations and requalify the process.

Variations in resistometer performance may in some instances give different resistance characteristics results for the indicator. In such cases, the manufacturer should, on request, give information on details of the relevant testing conditions.

If the user establishes data on the nominal population count or the *D*-value and these are outside the limits required by the relevant standards or outside the label information, the user is encouraged to seek information from the manufacturer to ensure that the same techniques, methods, and conditions are used to obtain the data (see references [20], [26], and [27]).

11.2 Nominal population of test organism

The manufacturer gives as part of the labeling information the nominal population of test organisms of each biological indicator. The requirements are given in the standards for the minimum number of microorganisms on a biological indicator or inoculated carrier to ensure a minimum resistance of the indicator. When tested, the number can be higher or lower than the labeled number because variations in testing procedures can influence the resulting data. The biological indicator manufacturer should be consulted to ensure that the same techniques and procedures are used. ISO 11138-1 requires the manufacturer to provide this information on request.

Spores, such as *Bacillus stearothermophilus*, may require a heat-shock procedure in order to obtain greater accuracy in counts. Several combinations of temperature and time have been used successfully. If the combination of time and temperature is different than that recommended by the manufacturer of the biological indicator, the specific conditions should be validated by the user. The mechanical treatment of the impregnated carrier and thus the microorganisms, during preparation of the aliquots, can influence the results (see reference [25]). Different laboratory practices and even variations in the performance of individual personnel can lead to different results.

The method for removing spores from the inoculated carrier should be validated and may include mechanical disintegration of the carrier or other methods, such as ultrasonication. If the user applies a different method from that recommended by the manufacturer, the method should be validated.

The fluid for disintegration should not influence the number of surviving microorganisms (e.g., not be a growth medium) and should not otherwise influence negatively the result by any inhibitory effect on the growth of the microorganism (see clause 12).

The user should follow the manufacturer's recommended procedures for recovery to ensure comparable results.

The sterilized fluid and the processed, impregnated carrier should be treated aseptically to avoid any microbial contamination or cross-contamination that could bias the results.

Attention should be given to the accuracy of the plate counts. The accuracy of plate counts is dependent on a variety of factors including dilution and pipetting error, calibration of pipetting devices, technician training, and the number of colony-forming units (cfu) per plate. It is generally accepted that plate counts should be between 30 cfu and 300 cfu per plate for greatest accuracy.

The International Standards limit deviations from the labeled nominal number. The user should note that if the nominal population of an indicator from a given batch or lot of indicators is being tested by the user, the deviations may exceed the limits given in the relevant part(s) of ISO 11138. This could be caused, for example, by different culture media used or different enumeration and counting techniques (see reference [23]).

11.3 *D*-value determination

11.3.1 General

If the user of the biological indicator chooses to verify the label claims, the same conditions as employed by the manufacturer should be followed, including the specific parameters employed for the relevant resistometer. ISO 11138-2 and ISO 11138-3 give minimum requirements for *D*-values for biological indicators for ethylene oxide and moist heat sterilization processes. A *D*-value can be estimated or calculated in many ways; the two principal ways are the direct enumeration method and the fraction-negative method. A comparison of different methods is given in reference [24]. The ISO 11138 series provides requirements for both types of method, which can be used in combination to estimate the *D*-value.

The main difference between the two approaches is given below.

a) Direct enumeration method:

   This method requires counting of colonies. Depending upon the type of the impregnated carrier and the properties of the microorganism, this often implies the use of mechanical degradation of the impregnated carrier (performed using aseptic techniques) with subsequent retrieval and counting of the total retrievable count of colony forming units on solid medium (e.g., distinct colonies on agar plates).

b) Fraction-negative method:

This method requires growth/no-growth determination and employs aseptic transfer of the intact inoculated carrier into the fluid culture medium after exposure to the whole sterilization process or after time intervals of the sterilization process (i.e., parts of the holding time). The transfer is performed without any mechanical, microbiological, or thermal influence on the inoculated carrier.

c) The survival-kill window:

This is based on a fraction-negative method, giving lower limits where all samples show growth and upper limits where none of the indicators shows growth (see ISO 11138-1).

11.3.2 Direct enumeration method

This method is also referred to as the “survivor curve method” and the “enumeration number method.” This method makes use of direct counting procedures (see ISO 11138-1) and should be performed on inoculated carriers (see annex A, Figure A.4).

For further details on procedures, see 11.2.

11.3.3 Fraction-negative method

There are several such methods in use, called fraction-negative, quantal, or most probable number analysis (MPN) methods. Growth or non-growth is observed relative to the number tested (see annex A, Figure A.4).

This International Standard gives the common reference method for the ISO 11138 series, which is the limited Spearman-Karber procedure (LSKP). Three other commonly used statistical methods, the Holcomb-Spearman-Karber procedure (HSKP), the Stumbo-Murphy-Cochran procedure (SMCP), and the limited Stumbo-Murphy-Cochran procedure (SMCP) may be used under particular conditions.

a) *Limited Spearman-Karber Procedure (LSKP):*

This procedure can be used if the successive exposure times differ by a constant time interval and if an identical number of replicates is exposed at each time interval. For example, exposures could be at 3 min, 5 min, 7 min, and 9 min, which represents a 2-min time interval. ISO 11138-1 specifies at least 20 replicates at each interval for the LSKP.

The average *D*-value is calculated from the equation:

$$D = \frac{U_{sk}}{\log_{10} N_0 + 0.2507}$$

where N_0 is the initial inoculum of test organisms per test sample, and

$$U_{sk} = U_k - \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{k-1} r_i$$

Examples of calculations are presented in annex C. For more information on LSKP, see ISO 11138-1 and reference [13].

b) *Holcomb-Spearman-Karber Procedure (HSKP):*

This method is similar to the LSKP but uses the generic formula which does not require use of the same number of replicates nor that constant time intervals be used.

The average *D*-value is calculated from the equation:

$$D = \frac{\hat{\mu}}{\log_{10} N_0 + 0.2507}$$

where $\hat{\mu} = \sum_{i=1}^{k-1} \mu_i$

Examples of calculations are presented in annex C.

c) *Stumbo-Murphy-Cochran Procedure (SMCP):*

The formula for the Stumbo-Murphy-Cochran procedure requires one result in the fraction-negative range, consisting of time (t), the number of units negative for growth (r), the number of replicates (n) at one exposure time within the fraction-negative range, and the initial number of microorganisms per replicate (N_0).

The *D*-value is calculated from the equation:

$$D = t / \left[\log_{10} N_0 - \log_{10} \left(\ln \frac{n}{r} \right) \right]$$

To obtain valid data using the Stumbo-Murphy-Cochran procedure, the *D*-value should be calculated as the average of at least three runs in the fraction-negative range in order to confirm reproducibility (see annex C).

d) Limited Stumbo-Murphy-Cochran Procedure (LSMCP):

A minimum of 40 biological indicators are required to attain data equivalent to that obtained using the LSKP. Not fewer than 50 biological indicators are required to obtain valid data. To attain a narrower confidence interval, negative numbers per sample number should be defined to be less than 0.9. With these restrictions (sample number ≥ 50, $r/n < 0.9$), the LSMCP is equivalent to the LSKP (see reference [24]).

11.4 Testing equipment

Particular attention should be given to the kind of resistometer used. The ISO 11138 series gives requirements for special resistometers to be used in order to comply with the requirements of the standards. Manufacturers of biological indicators shall comply with the relevant standards in the ISO 11138 series if they declare conformity to these standards.

A pilot-plant sterilizer or production sterilizer would only provide a figure for the microbial log reduction. Attention should be given to the set of parameters and the number of vacuum pulses as well as the depth of vacuum.

All technical equipment, including automatic or adjustable pipettes, should be periodically calibrated and/or controlled.

The control and maintenance of technical equipment should be documented.

12 Culture conditions

12.1 General

Manufacturers of biological indicators are required by ISO 11138-1 to provide information to the user as to the culturing conditions (i.e., incubation temperature, incubation period, and choice of growth medium). If the user employs other culturing conditions, these should be validated by the user.

The procedures should be performed in a laboratory area defined for this purpose, giving proper attention to aseptic technique and good laboratory practices. It is a good practice to include negative controls with each assay performed when using general laboratory areas for these assays. If a defined area cannot be found for this purpose, or if there is any risk of cross-contamination, the procedure should be performed in a defined critical zone (e.g., a sterile bench or biosafety cabinet with no air exchange between the critical zone and surrounding areas where biological indicators or microorganisms of the same or similar growth properties are being manufactured, packaged, or otherwise handled).

Culture conditions recommended by the manufacturer of the biological indicator should be followed. If incubation conditions other than those recommended by the manufacturer are employed, they should be validated to determine their effect on the performance of the biological indicator.

For validation purposes, the incubation conditions should be carefully considered. The user is advised to seek information in available International Standards, such as the ISO 11737 series (see clause 2 and reference [9]).

12.2 Incubation temperature

The labeling of the biological indicator should always be consulted with regard to the optimum incubation temperature. Failure to incubate biological indicators at the appropriate incubation temperature may invalidate the results of testing.

Test organisms that have been processed by sterilization may exhibit increased sensitivity to variations in incubation temperature. Some test organisms may exhibit increased recovery at incubation temperatures below the optimum incubation temperature and decreased recovery at incubation temperatures above the optimum incubation temperature. In general, biological indicators of *Bacillus stearothermophilus* may be incubated at temperatures in the range of 55 °C to 60 °C, and biological indicators of *Bacillus subtilis* may be incubated at temperatures in the range of 30 °C to 37 °C or according to the specifications provided by the manufacturer.

12.3 Incubation period

The incubation period required may vary with the nature of the biological indicator and sterilization process. The labeling of the biological indicator and other information provided by the manufacturer should be consulted in this regard.

For non-standard or new sterilization processes not covered by the current International Standards, the incubation period of the biological indicator should be validated against current national requirements. If there are no current national requirements, a period of 14 days for sterility testing may be used for the validation of incubation period for biological indicators (see reference [9]).

Retrospective evaluation of incubation period data is useful as a basis for the recommended incubation period. Retrospective validation is particularly useful if it includes documented evidence on time intervals from start of culturing until end of any response showing growth of the indicator organism. Where retrospective validation produces information on response times for indicators that have been exposed to different kinds of sterilizers and sterilizer programs employing principally the same sterilization method, a sound basis is provided for choosing the routine incubation period used in a wide variety of sterilizers.

For industrial purposes it may be useful to validate a reduced incubation period for a particular biological indicator and sterilization process. This should be determined through consultation with the appropriate regulatory authorities. A Technical Report covering validation of incubation period is under preparation.

12.4 Choice of growth medium

Most manufacturers of biological indicators either provide culturing medium directly or provide information regarding the preparation of a suitable culturing medium. The culturing medium employed by different manufacturers may vary significantly; thus, it is important to follow the biological indicator manufacturer's recommendations.

For validation purposes of biological indicator incubation period in particular, the inherent variability of the digest employed to prepare most culturing media makes it advisable to screen the performance of several medium lots and to reserve suitable quantities of lots found to provide the desired growth performance. This would allow comparison with new lots.

Selection of a suitable culturing medium requires consideration of many variables, such as the pH of the culturing medium and the presence of inhibitory substances such as salts, pH indicators, or antibiotics. Other substances in the culturing medium may affect the recovery of sterilizing agent-stressed test organisms. Users should not overprocess the culture medium, as extended sterilization may induce changes that can affect its growth-promoting properties. The ability of the culturing medium to promote the growth of low numbers of microorganisms should be demonstrated (see references [16] to [19] and [28]).

Each lot of growth medium should be checked by a suitable growth promotion test and compared with a lot previously used to determine lot-to-lot consistency.

13 Third-party requirements

13.1 General

When agreement cannot be reached between the user and the manufacturer of biological indicators, third parties can provide impartial testing results based on the requirements of the ISO 11138 series.

A third-party testing facility could be a testing laboratory that has been accredited or which has an otherwise officially recognized quality assurance system for the service (see reference [3]).

Third-party facilities should employ the test equipment and test methods, including all parallel and repeated tests that are required by the relevant International Standards.

A testing laboratory operating as a third party for the purpose of testing biological indicators according to the ISO 11138 series should document that the scope of the accreditation covers the specific testing required by those International Standards. In addition, a testing laboratory operating as a third party is required to be independent of any biological indicator manufacturer's facilities and should not be owned by a biological indicator manufacturer (see reference [2]). The accuracy of the methods should be documented.

Test reports should be reviewed and signed for approval by a designated person. Accredited laboratory test reports should be signed by the person who is recognized by an accreditation body as competent to sign such reports.

For resistance test methods, subclause 5.1.2 of ISO 11138-1:1994 requires a combination of two or more of the following methods:

a) survivor curve (direct enumeration method);

b) fraction-negative analysis; or

c) survival-kill window.

All the required runs, as well as the required number of replicates for each run, are given as minimum numbers.

13.2 Minimum requirements for replicates and total number of biological indicators

Table 1—Presentation of minimum number of biological indicators required for testing according to ISO 11138-1

ISO 11138-1	Minimum number of replicates	Minimum number of parallels	Minimum number of exposure conditions	Minimum total number of biological indicators
Annex B survivor curve	4	1	5	20
Annexes C and D LSKP	20	1	6	120
Annex E survival-kill window	50	1	2	100
Nominal population	4	—	—	4
Minimum total number				124

ISO 11138-1 requires a total of at least 20 biological indicators for the counting procedures given in annex B, with at least five exposure conditions and four replicates for each condition. Annexes C and D cover minimum requirements for the LSKP, with a total of at least 120 biological indicators. A minimum of six graded exposure conditions are used with 20 replicates each. One exposure condition should result in all positive biological indicators, as well as the exposure preceding this exposure. There shall be at least two sequential exposures that result in no positive biological indicators. There should be a minimum of two intermediate exposures that result in fractional responses. The survival-kill window characterization, given in reference [24], requires a total of 100 biological indicators with 50 replicates at the two conditions. The resistance characteristics are determined according to ISO 11138-1, which requires employment of at least two methods out of the three mentioned. This implies the use of at least 124 or 144 or 224 biological indicators respectively, according to the methods chosen.

At least two *D*-value determinations are required to estimate the *z*-value for moist heat sterilization processes according to 11138-3:1995, subclause 9.4.

ISO 11138-1 requires four replicates for the nominal number-counting procedures (nominal number determination).

13.3 Test equipment

A testing laboratory that performs tests according to the ISO 11138 series needs to apply the required test equipment, including the relevant resistometer. The documentation of parameter readouts from the resistometer should be detailed enough to verify that the requirements for the process parameters have been met.

For further information, see 11.4.

14 Personnel training

Personnel responsible for the placement, retrieval, testing, and all other handling of biological indicators need to be suitably trained. This training should be documented, and the adequacy of the training should be periodically assessed. There should be written procedures for the testing and handling of the biological indicators as well as for supporting activities such as preparing and sterilizing culture medium.

When aseptic techniques are used, particular attention should be given to training personnel in these techniques.

15 Storage and handling

The vendor or supplier is responsible for shipment or transport to the user and should ensure that temperature variations that occur during transport do not have an adverse effect on the labeled resistance characteristics. The vendor or supplier should make agreements with the user on means of transportation, to ensure that conditions are adequate to retain the performance characteristics of biological indicators during the transportation.

The recommendations of the manufacturer with regard to the storage and handling of biological indicators should always be followed. Failure to follow these recommendations could adversely affect the integrity and performance of the biological indicator and lead to incorrect assumptions regarding the efficacy of the sterilization process. In general, biological indicators should always be maintained in their protective packaging until ready for use. A

biological indicator is delivered ready to use and in a packaging system that protects it from extraneous microbiological influences. Biological indicator storage should take into account temperature, relative humidity, chemical influences, and light. The latter is a factor with self-contained biological indicators, where photodegradation of the medium may be a concern. Biological indicators usually can be stored at ambient temperatures. Biological indicators should be kept protected from light.

Biological indicators consisting of non-hazardous microorganisms can be handled without restriction, and shipment and transport should follow international rules for transport of non-hazardous microorganisms.

16 Disposal of biological indicators

According to ISO 11138-1, the biological indicator manufacturer is required to provide disposal instructions. Inactivated biological indicators can be disposed as household waste. Expired or unused indicators can also be disposed as household waste if the microorganism is of a non-hazardous nature. However, manufacturers' disposal instructions, which often require sterilization prior to disposal, should be followed.

NOTE—National regulations may define biological indicators as hospital waste, and disposal of these biological indicators may be covered by regional or national regulations.

Annex A
(informative)

Microbiological inactivation kinetics and enumeration techniques

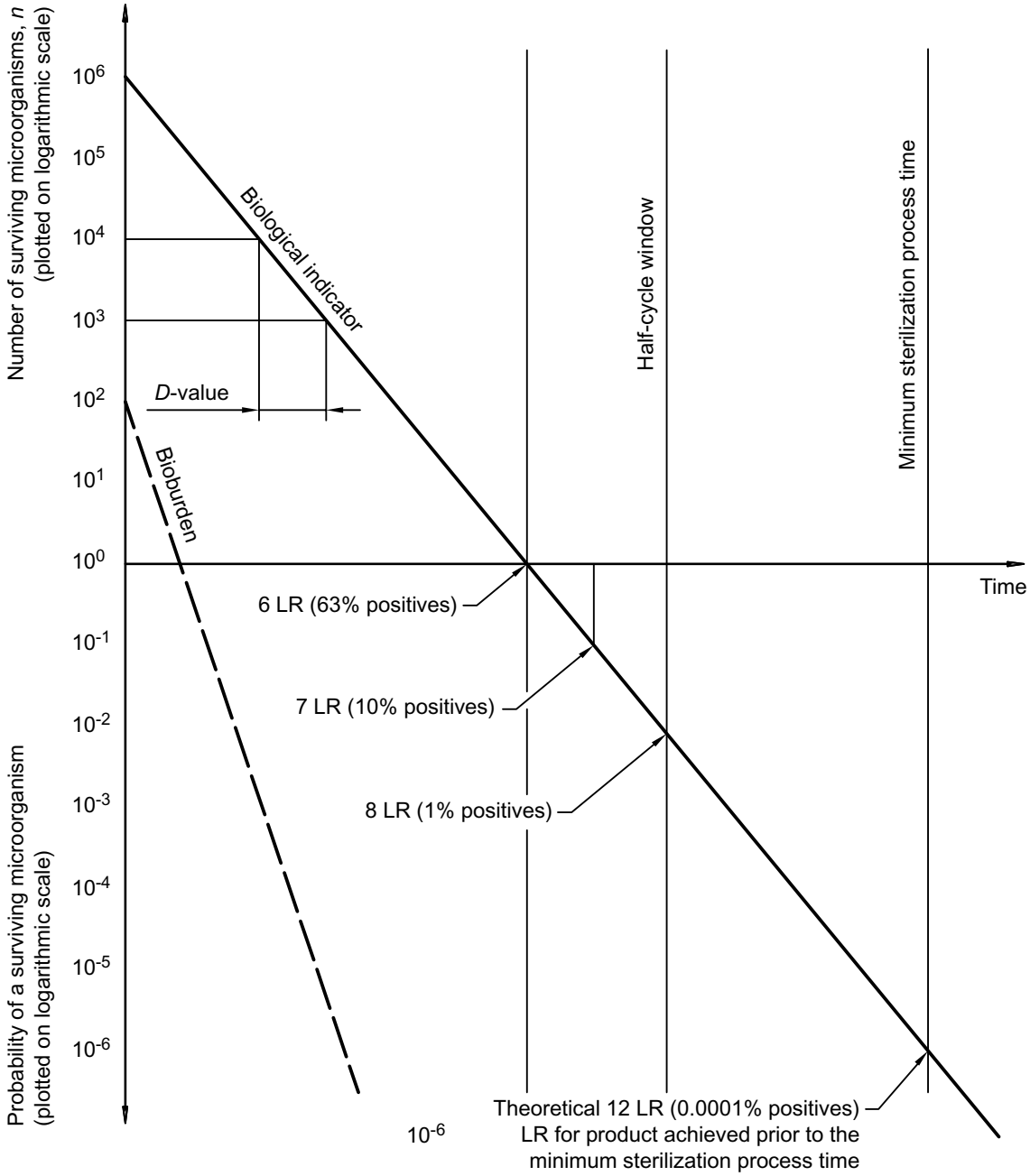


Figure A.1—Examples of relationship between the biological indicator and the product bioburden in a reference microorganism method, where LR is the "log reduction" (see 3.8)

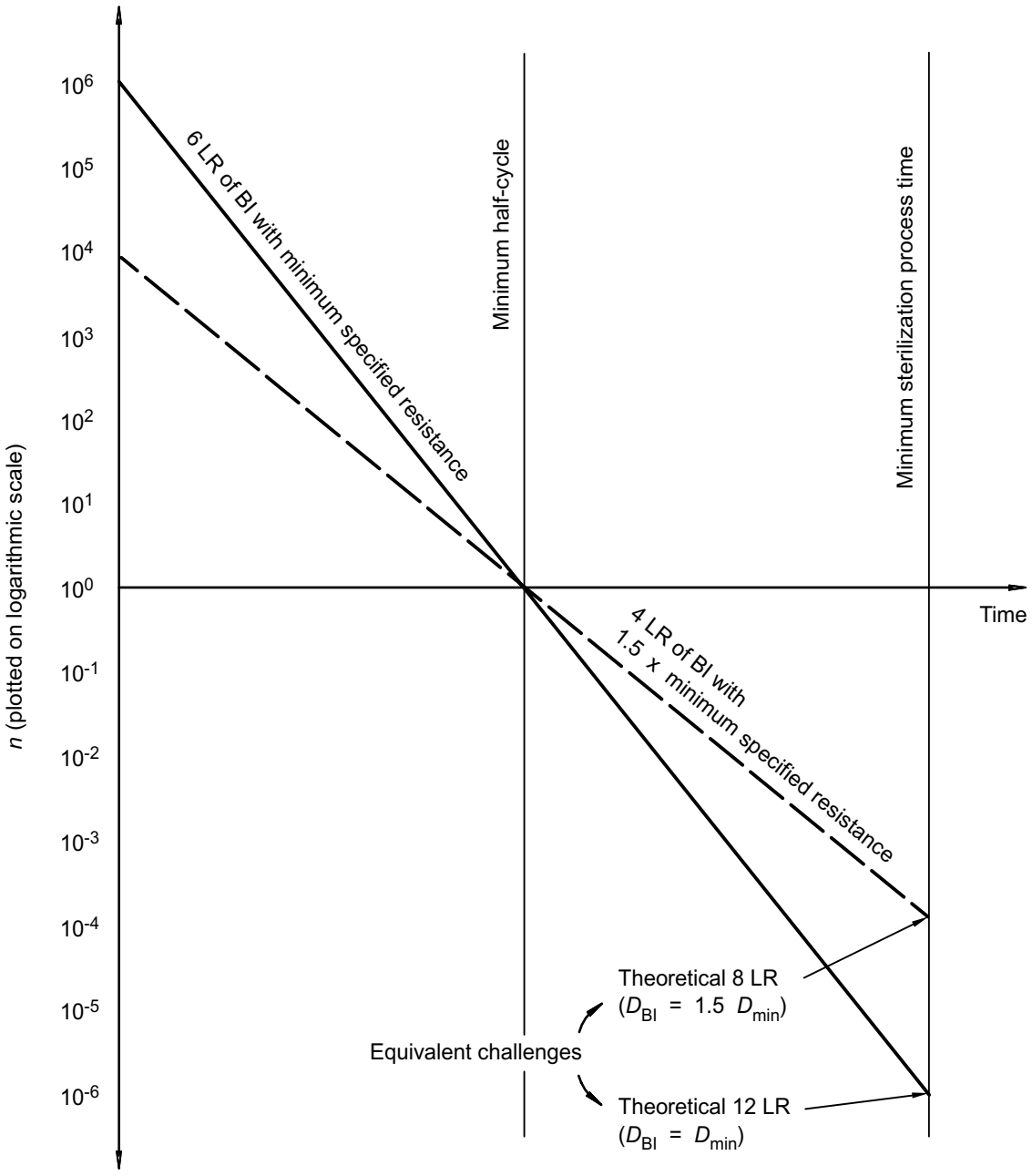


Figure A.2—Examples of equivalent biological challenges for the fractional cycle method

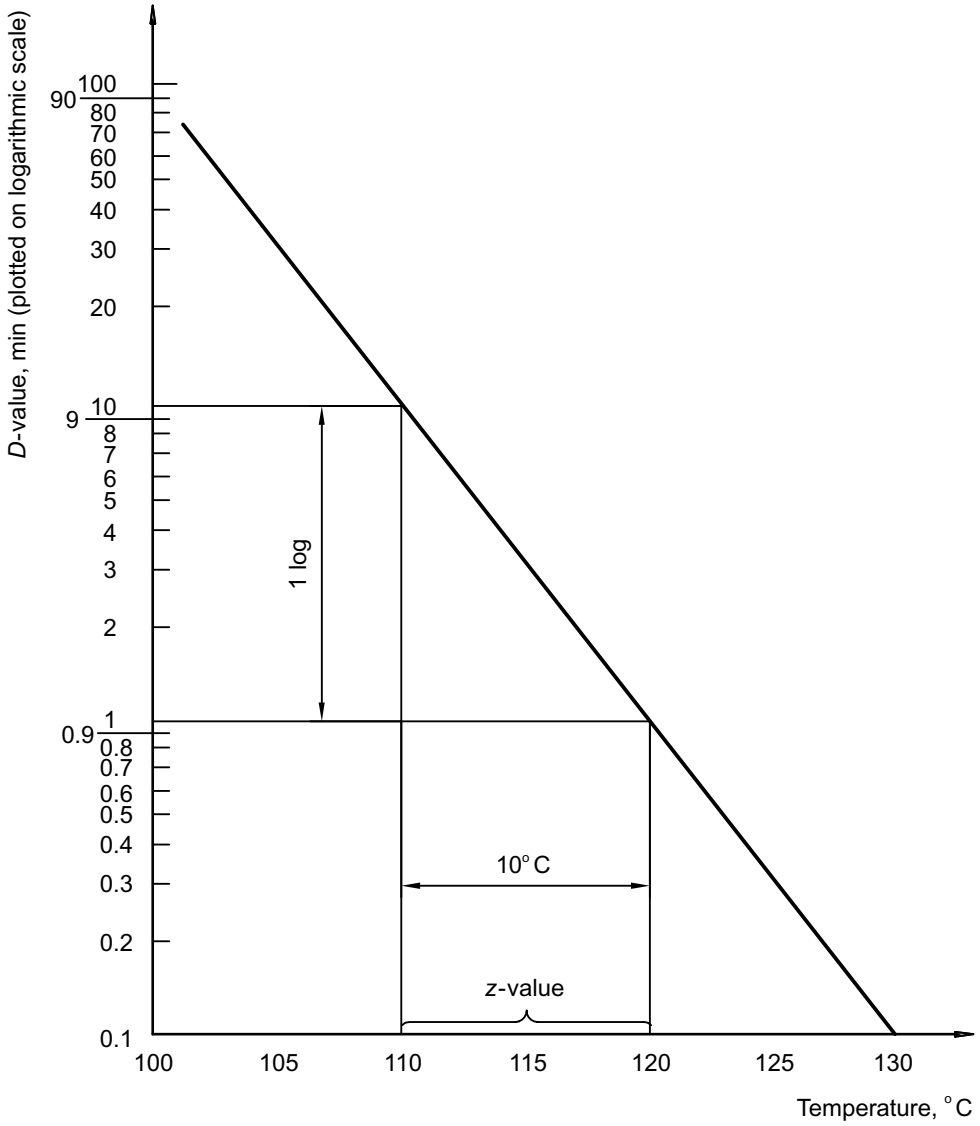


Figure A.3—*Z*-value determination

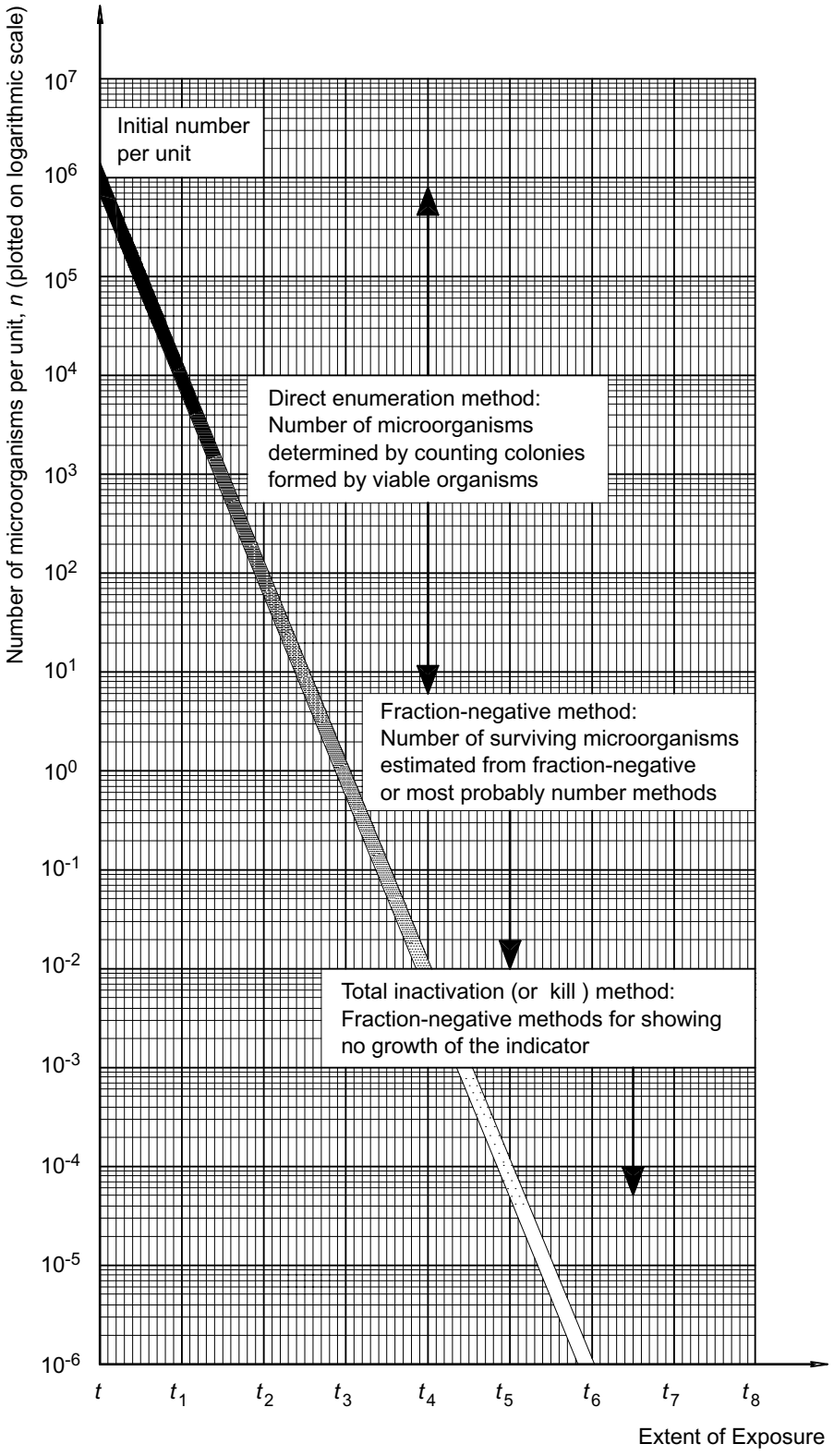


Figure A.4—Areas for *D*-value determination methods under uniform conditions

Annex B
(informative)

Process challenge devices

B.1 General

A process challenge device may have several configurations and uses. It is an object that simulates the worst-case conditions as they are given by the sterilizing agent(s) for the items to be sterilized. The commercially available process challenge device shall represent a similar or greater challenge to the user's sterilization process than would be represented by the load.

The device is constituted so that a biological indicator can be arranged in the place most difficult for the sterilizing agent to reach. The design of the process challenge device depends on the kind of goods to be sterilized and the sterilization procedure. The biological indicator should not interfere with the function of the process challenge device.

In some process challenge devices, an inoculated carrier may be used in place of a biological indicator.

B.2 Helices

The helix consists of a coiled tube with a gas-tight capsule for the inoculated carrier at one end, intended for challenging sterilizing agent penetration into long hollow instruments.

NOTE—National standards for specific sterilizers may cover requirements for such helices.

B.3 Standard test packs

Standard test packs are used in large steam sterilizers for porous loads to check that rapid and even penetration of steam into the pack is attained at the levels at which the process variables are set.

The standard test pack is comprised of porous sheets wrapped in a particular configuration with biological indicators inside. They are designed to test the efficacy of the steam sterilization process for porous loads.

NOTE—National standards for specific sterilizers may cover requirements for such standard test packs.

B.4 User's process challenge devices

This type of process challenge device is specially designed to meet the criteria for the process challenge location(s) in the load. The packaging and design should reflect the standardized load to be examined and varies with the load. The user's process challenge device serves as a "dummy" to replace the actual goods for this location and to allow removal of biological indicators without destroying the goods to be sterilized. The user may need one or several devices to cover the process challenge location(s).

B.5 Biological test packs

This is a common classification of commercially available process challenge devices that has a specified level of resistance to a biological indicator placed in a defined position. Biological test packs can be reusable or single-use items, depending on the materials used and the process parameters.

Annex C
(informative)

Formulae for fraction negative methods for *D*-value calculations

NOTE—The worked examples cover the methods referred to in 11.3 *D*-value determination: HSKP, LSKP, and LSMC.

C.1 Holcomb-Spearman-Karber Procedure (HSKP) Calculation of *D*-value

In the calculation given below, the data for the example described in 11.3.3 is codified in Table C.1.

Table C.1—Examples of data collected for HSKP

Time of exposure to sterilizing agent	Number of test samples exposed	Number of test samples showing no growth
t_1	n_1	r_1
t_2	n_2	r_2
t_3	n_3	r_3
t_4	n_4	r_4
t_5	n_5	r_5
t_6	n_6	r_6
t_7	n_7	r_7

At t_1, the shortest exposure time to sterilizing agent, all test samples show growth. The times of exposure to sterilizing agent t_2 through t_5 are increasing exposure times in the fraction-negative area (see annex A). Exposure times t_6 and t_7 are the two exposure times at which all samples show no growth.

For times of exposure to sterilizing agent, t_1 to t_6, the factors χ and γ are calculated as shown:

$$\chi_i = \frac{t_i + t_{i+1}}{2}$$

$$\gamma_i = \frac{r_{i+1}}{n_{i+1}} - \frac{r_i}{n_i}$$

r is the number of test samples out of the number exposed (n_i) showing no growth at exposure time t_i.

At t_1, all test samples show growth and so $\gamma_i = \frac{r_{i+1}}{n_{i+1}}$

From the calculated values of χ_i and γ_i above, the value μ_i can be calculated for each exposure time (t_i), as follows:

$$\mu_i = \chi_i \gamma_i$$

The mean time to attain no growth, $\hat{\mu}$, from any of the test samples can then be calculated as the sum of μ_i for each exposure time t_1 to t_6:

$$\hat{\mu} = \sum_{i=1}^{i=6} \mu_i$$

Where the interval between exposure times (d) is constant and the same number of test samples (n) is used at each exposure time, the mean to attain no growth ($\hat{\mu}$) can be calculated from the equation

$$\hat{\mu} = t_i - \frac{d}{2} - \frac{d}{n}\sum_{i=1}^{i=6} r_i$$

The mean D value ($\bar{D}$) can be calculated from the equation:

$$\bar{D} = \frac{\hat{\mu}}{0.2507 + \log_{10} N_0}$$

where N_0 = the initial inoculum of test organisms per test sample.

Calculation of 95 % confidence interval:

$$D_{calc} = D + 2\sqrt{V}$$

Calculation of variance:

$$V = a\left(\frac{2.3026}{0.5772 + \ln N_0}\right)^2$$

Calculation of a for variance formula:

$$a = 0.25\sum_{i=2}^{i=6}(t_{i+1} - t_{i-1})^2 r_i \frac{n_i - r_i}{n_i^2 (n_{i-1})}$$

Table C.2—Examples of data with non-constant time intervals and non-constant number of samples

Exposure time to sterilizing agent t	**Number of test samples exposed** n	**Number of test samples showing no growth** r_i
t_1 10	n_1 20	r_1 0
t_2 18	n_2 19	r_2 4
t_3 28	n_3 21	r_3 8
t_4 40	n_4 20	r_4 12
t_5 50	n_5 20	r_5 16
t_6 60	n_6 20	r_6 20
t_7 70	n_7 20	r_7 20

Example of calculations using the HSKP:

a) Calculate χ_i and γ_i (for each exposure)

$$\chi_1 = \frac{t_1 + t_{1+1}}{2} = \frac{10 + 18}{2} = 14$$

$$\chi_2 = \frac{18 + 28}{2} = 23$$

$$\chi_3 = \frac{28 + 40}{2} = 34$$

$$\chi_4 = \frac{40 + 50}{2} = 45$$

$$\chi_5 = \frac{50 + 60}{2} = 55$$

$$\chi_6 = \frac{60 + 70}{2} = 65$$

$$\gamma_1 = \frac{r_{1+1}}{n_{1+1}} - \frac{r_1}{n_1} = \frac{4}{19} - \frac{0}{20} = 0.21$$

$$\gamma_2 = \frac{8}{21} - \frac{4}{19} = 0.17$$

$$\gamma_3 = \frac{12}{20} - \frac{8}{21} = 0.22$$

$$\gamma_4 = \frac{16}{20} - \frac{12}{20} = 0.2$$

$$\gamma_5 = \frac{20}{20} - \frac{16}{20} = 0.2$$

$$\gamma_6 = \frac{20}{20} - \frac{20}{20} = 0$$

Note that for the calculations of γ_4 and γ_5, both γs = 0.2. This happened because the number of test samples showing no growth increased at a constant rate in this example.

b) Calculate r_i for each exposure time (t_i):

$\mu_i = \chi_i \gamma_i$

$\mu_1 = \chi_1 \gamma_1 = 14 \times 0.21 = 2.94$

$\mu_2 = 23 \times 0.17 = 3.91$

$\mu_3 = 34 \times 0.22 = 7.48$

$\mu_4 = 45 \times 0.2 = 9.0$

$\mu_5 = 55 \times 0.2 = 11.0$

$\mu_6 = 65 \times 0 = 0$

c) Calculate the mean time to attain no growth, $\bar{\mu}$

$$\bar{\mu} = \sum_{i=1}^{i=6} \mu_i$$

$$\bar{\mu} = 2.94 + 3.91 + 7.48 + 9.0 + 11.0 + 0 = 34.33$$

d) Calculate the mean *D*-value, $\bar{D}$

$$\bar{D} = \frac{\bar{\mu}}{0.2507 + \log_{10} N_0}$$ where N_0 = initial population of 1×10^5,

$$\bar{D} = \frac{34.33}{5.2507} = 6.54$$

e) Calculate the upper 95 % confidence level for $\bar{D}$ (D_{calc})

$$D_{calc} = \bar{D} + 2\sqrt{V}$$

$$V = a\left(\frac{2.3026}{0.57722 + \ln N_0}\right)^2$$

$$a = 0.25\sum_{i=2}^{i=6}(t_{i+1} - t_{i-1})^2\left[r_i \frac{n_i - r_i}{n_i^2 (n_{i-1})}\right]$$

First find *a* by performing the above calculation for each t_i and summing all results:

$$a = 0.25 \times \left\{\left[(28-19)^2 \times 4\frac{19-4}{361 \times 18} = 2.9917\right] + \left[(40-18)^2 \times 8\frac{21-8}{441 \times 20} = 5.7070\right] + \left[(50-28)^2 \times 12\frac{20-12}{400 \times 19}\right.\right.$$

$$\left.\left. = 6.1137\right] + \left[(60-42)^2 \times 16\frac{20-16}{400 \times 19} = 3.3684\right] + \left[(70-50)^2 \times 20\frac{20-20}{400 \times 19} = \frac{0.0000}{18.1808}\right]\right\}$$

$$a = 0.25 \times 18.1808 = 4.5452$$

$$V = 4.5452\left(\frac{2.3026}{0.57722 + \ln (1 \times 10^5)}\right)^2$$

$$= 4.5452\left(\frac{2.3026}{0.57722 + 11.513}\right)^2$$

$$= 4.5452 \times (0.19045)^2$$

$$= 4.5452 \times 0.03627$$

$$= 0.1649$$

$$D_{calc} = D + 2\sqrt{V} = 6.54 + 2\sqrt{0.1649} = 6.54 + 2 \times 0.4061 = 7.35$$

C.2 Limited Spearman-Karber Procedure (LSKP)

Table C.3—Examples of data with constant time intervals and constant number of samples

Exposure time to sterilizing agent t	Number of test samples exposed n	Number of test samples showing no growth r_i
t_0 18 ($U_{1\text{-}1}$)	n_0 20	r_0 0 ($r = 0$)[a]
t_1 20 (U_1)	n_1 20	r_1 0 ($r = 0$)
t_2 22	n_2 20	r_2 1
t_3 24	n_3 20	r_3 7
t_4 26	n_4 20	r_4 15
t_5 28	n_5 20	r_5 19
t_6 30 (U_k)	n_6 20	r_6 20 ($r = n$)[a]
t_7 32	n_7 20	r_7 20 ($r = n$)

[a] The test is valid if there are no negative units, i.e., no negative replicates ($r = 0$), at the exposure preceding U_1 and all negative replicates, i.e., all replicates showing growth ($r = n$), at the exposure subsequent to U_k.

$$U_{sk} = U_k - \frac{d}{2} - \frac{d}{n}\sum_{i=1}^{k-1} r_i \qquad \text{(see 11.3.3)}$$

$U_k = 30$

$d = 2$

$n = 20$

$N_0 = 1 \times 10^6$

$$U_{sk} = 30 - \frac{2}{2} - \frac{2}{20} \times (0 + 0 + 1 + 7 + 15 + 19) = 24.8$$

$$D = \frac{U_{sk}}{0.2507 + \log_{10} N_0} = \frac{24.8}{6.2507} = 3.97 \text{ min}$$

$$V = \frac{d^2}{n^2(n-1)} \times \sum_{i=1}^{k-1} r_i\,(n - r_i) = \frac{4}{7600} \times [(1 \times 19) + (7 \times 13) + (15 \times 5) + (19 \times 1)] = 0.1074$$

$$SD = \sqrt{V} = 0.3277$$

with a 95% confidence interval for D:

$$D \text{ lower limit} = \frac{U_{sk} - 2SD}{\log_{10} N_0 + 0.2507} = \frac{24.144}{6.2507} = 3.86 \text{ min}$$

$$D \text{ upper limit} = \frac{U_{sk} - 2SD}{\log_{10} N_0 + 0.2507} = \frac{25.455}{6.2507} = 4.07 \text{ min}$$

C.3 Limited Stumbo-Murphy-Cochran Procedure (LSMCP)

Exposure time = 24

r = 37

n = 100

$N_0 = 1 \times 10^6$

$D = t(\log_{10} A - \log_{10} B)$ where:

t = exposure time

$\log_{10} A = \log_{10}$ of initial population (N_0) per replicate

$\log_{10} B = \log_{10}$ of population after exposure time (t) or

$\log_{10} B = \log_{10} (\ln n/r)$ or $\log_{10} [2.303 \log_{10} (n/r)]$ where:

n = number of replicates per exposure time

r = number of units sterile or showing no growth

$$D = \frac{t}{\log_{10} N_0 - \log_{10} (\ln \frac{n}{r})} = \frac{24}{6 - \log_{10} (\ln \frac{100}{37})} = 4.00$$

with a 95% confidence interval:

If $n \cdot \frac{r}{n} \cdot \frac{n-r}{n}$ is not less than 9, then the 95% confidence interval can be calculated using the following equations:

$$\frac{r}{n} \pm 1.96 \sqrt{\frac{r}{n} \cdot \frac{1 - r/n}{n}} = 0.37 \pm 0.095$$

$$D \text{ lower limit} = \frac{24}{6 - \log_{10} \times \ln \left(\frac{1}{0.37 + 0.095} \right)} = 3.92$$

$$D \text{ upper limit} = \frac{24}{6 - \log_{10} \times \ln \left(\frac{1}{0.37 + 0.095} \right)} = 4.08$$

Annex D
(informative)

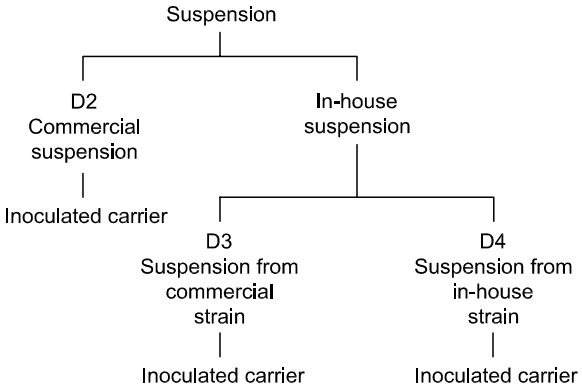
Examples of documentation of biological indicators collected by the user

D.1 General

D.1.1 Sources of microorganism

There are different sources of microorganisms for biological indicators. Biological indicators may be delivered from a manufacturer as a ready-to-use system with resistance characteristics in accordance with the ISO 11138 series. Consult the main document for commercially available biological indicators. Commercially available suspensions of microorganisms delivered from a manufacturer of biological indicators may be used to inoculate products, thereby using the product as the carrier. Alternatively, an in-house-made suspension could be produced from a commercially available strain. In particular circumstances, microorganisms isolated from the production plant (in-house isolates) may represent the most resistant microorganisms likely to be found in or on the products to be sterilized. In such cases, biological indicators can be produced using the relevant microorganism (see 7.3).

Biological indicators may be prepared from microorganisms of one of three provenances:



D.1.2 Documentation

A list of relevant documents may include:

a) work instructions for production and handling of microbiological strains, preparation of in-house suspensions, impregnation of carriers, and handling of impregnated carriers and in-house biological indicators;

b) work instructions for handling of the microbiological systems after sterilization-cycle processing;

c) protocols for validation studies and results.

The user is advised to define basic elements such as:

d) the method of sterilization to be used in the routine production of products, e.g., moist heat, dry heat, gaseous, or other sterilization method; and

e) which of the critical sterilization parameters is measured directly during the routine production of products, e.g., time, temperature, pressure, etc.

NOTE—This list of elements to document is not necessarily exhaustive.

D.2 Commercially available suspension

When using commercially available suspensions, the following elements should be documented:

a) name of manufacturer of suspension;

b) name and identification of the microorganism;

c) origin of the strain/reference to a recognized culture collection number;

d) number of microorganisms to be detected per defined volume of suspension;

e) suspending medium;

f) lot number or other means of identification;

g) resistance characteristics as given by the manufacturer of the suspension;

h) recommended culture conditions for retrieval of viable microorganisms from the suspension, e.g., time, temperature, and growth medium;

i) storage conditions;

j) stability information (expiry date or equivalent).

NOTE—This list of elements to document is not necessarily exhaustive.

D.3 Suspension from a commercially available strain

When using suspensions prepared from a commercially available strain, the following elements should be documented:

a) name of manufacturer or supplier of the strain;

b) name of the microorganism;

c) reference to a recognized culture collection number;

d) the manufacturer's instructions for handling of the strain;

e) relevant information on processing elements for preparing the suspension from the strain;

f) storage conditions;

g) means of identification of the stored and processed strain/lot No.;

h) stability information.

NOTE—This list of elements to document is not necessarily exhaustive.

D.4 Suspension from in-house isolates

When preparing a suspension from in-house isolates, i.e., a microbiological strain isolated from within the main production area, the following elements should be documented:

a) place of isolation;

b) date of retrieval;

c) isolation method/retrieval method;

d) identification of the microorganisms;

e) culture conditions, e.g., culture medium, incubation temperature and time;

f) method of processing to a suspension;

g) suspension fluid;

h) means of identification of the suspension/lot No.;

i) resistance determination;

j) storage conditions;

k) stability information.

NOTE—This list of elements to document is not necessarily exhaustive.

D.5 Inoculated carriers

D.5.1 General

The carrier material should represent the carrier material to be sterilized in the main production. The carrier material can be a fluid or a solid surface that represents the product to be validated for a sterilization process. The carrier material is chosen as a rigorous challenge carrier based on validation.

D.5.2 Documentation of fluid carrier materials

Some relevant elements for the documentation of use of fluids as carrier material are listed below:

a) method used to ensure even dispersion of microorganisms in the suspension before use;

b) description of the carrier material, e.g., pH value of the carrier material;

c) description of the fluid containers;

d) method used for inoculation of the suspension into the fluid;

e) methods used to ensure even dispersion of microorganisms in the test vials before resistance characteristics testing is carried out;

f) number of microorganisms in the inoculated fluid;

g) storage of the test containers prior to testing;

h) retrieval method of the microorganisms after testing;

i) culture conditions for microorganisms after testing.

NOTE—This list of elements to document is not necessarily exhaustive.

D.5.3 Documentation of solid carrier materials

Some relevant elements for documentation of use of solid surfaces as carrier material are given below:

a) methods to ensure even dispersion of microorganisms in the suspension before use;

b) description of the carrier material, e.g., pH value of the carrier material;

c) method used for inoculation of the suspension onto the carrier;

d) procedure for control of even dispersion of microorganisms on the carrier;

e) number of microorganisms on the carrier;

f) storage conditions of the inoculated carrier;

g) method(s) of retrieval of the microorganisms;

h) culture conditions for retrieved microorganism.

NOTE—This list of elements to document is not necessarily exhaustive.

D.5.4 Documentation of inoculated carriers used for *D*-value determinations

Inoculated carriers, whether used as-is or with a packaging system, such as fluids in vials, that are used for *D*-value determinations or other resistance characteristics studies, have their characteristics described by the data obtained. The methods chosen have an impact on these data.

For comparing *D*-value determinations of carriers inoculated in-house by the user with commercially available biological indicators complying with the ISO 11138 series, the *D*-value determinations should comply with the relevant requirements of the ISO 11138 series (see 11.3).

Some basic elements should be documented:

a) description of resistometer, pilot plant sterilizer, or production sterilizer;

b) description of methods for direct physical measurement, such as number and position of temperature probes;

c) initial population determination;

d) methods used for determination of resistance characteristics, such as survival-kill-window, most probable number, or counting procedures;

e) number of parallels and number of runs of the resistance determination;

f) retrieval method of the microorganisms;

g) culture conditions;

h) recommended use of the validation or other studies of the sterilization process in the routine production.

NOTE—This list of elements to document is not necessarily exhaustive.

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