List of Titles

American National Standard

ANSI/AAMI ST:59:1999

Sterilization of health care products—Biological indicators—Part 1: General

AAMI Association for the Advancement of Medical Instrumentation

American National Standard ANSI/AAMI ST59:1999

Sterilization of health care products—Biological indicators—Part 1: General

Developed by
Association for the Advancement of Medical Instrumentation

Approved 13 September 1999 by
American National Standards Institute, Inc.

Abstract: This standard specifies general production, labeling, and performance requirements for the manufacture of biological indicators and suspensions intended for use in the validation and monitoring of sterilization cycles.

Keywords: carrier, culture, growth, pack, organism, resistance, survival, value

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Association for the Advancement of Medical Instrumentation

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This American National Standard was developed by the Biological Indicators Working Group under the auspices of the AAMI Sterilization Standards Committee.

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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.

Acknowledgment

The Biological Indicators Working Group would like to thank Dr. Gary Graham, PhD for his contribution to the development of this standard during his tenure as cochair of the Biological Indicators Working Group.

Foreword to the American National Standard

This American National Standard, ANSI/AAMI ST59, *Sterilization of health care products—Biological indicators—Part 1: General*, specifies general production, labeling, and performance requirements for the manufacture of biological indicators. Other parts are available, including:

Part 2: Biological indicators for ethylene oxide sterilization

Part 3: Biological indicators for moist heat sterilization

These standards replace the previous editions of ANSI/AAMI Standards covering biological indicators (ANSI/AAMI ST21:1986/(R)1994 and ANSI/AAMI ST19:1986/(R)1994).

These American National Standards were developed by the AAMI Biological Indicators Working Group under the auspices of the AAMI Sterilization Standards Committee.

These American National Standards are based on the International Organization for Standardization (ISO) series of standards for biological indicators (ISO 11138 series) developed by Working Group 4 (WG 4), *Biological indicators* of ISO Technical Committee 198, *Sterilization of health care products.* The U.S. member body of ISO, the American National Standards Institute (ANSI), held the international secretariat of ISO/TC 198 and assigned administration of this technical committee to AAMI.

AAMI also coordinated U.S. participation in ISO/TC 198 and in WG 4 through the U.S. Technical Advisory Group (TAG) for ISO/TC 198. Specific participation on WG 4 was coordinated by the U.S. Sub-TAG for ISO/TC 198/WG 4 (AAMI Biological Indicators Working Group).

These American National Standards contain significant national deviations from the corresponding ISO standards. All substantive national deviations are described in annex H, and a rationale for each change is provided.

Annexes A through G to this standard are normative. Annex H to this standard is informative.

As used within the context of this standard, “shall” indicates requirements strictly to be followed in order to conform to the standard; “should” indicates that among several possibilities one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action is discouraged but not prohibited; “may” is used as a statement of possibility and capability. “Must” is used only to describe “unavoidable” situations.

NOTE—This foreword does not contain provisions of the American National Standard, *Sterilization of health care products—Biological Indicators—Part 1: General* (ANSI/AAMI ST59:1999), but it does provide information about the development and intended use of the document.

Introduction

This standard specifies general production, labeling, and performance requirements for the manufacture of biological indicators (BIs) intended for use as monitors of sterilization cycles. The procedures and methods described in this standard should be carried out by suitably trained personnel.

BIs are not intended for use in any process other than that specified by the manufacturer on the labeling. The use of an inappropriate BI can give misleading results. BIs are used to test the effectiveness of sterilization processes and equipment.

The performance of a BI can be adversely affected by the conditions of transportation, storage prior to use, the methods of use, or the techniques employed after exposure to the process. For these reasons, the recommendations of the manufacturer for storage and use should be followed, and BIs should be transferred to the specified recovery conditions as soon as possible after exposure to the process. BIs should not be used beyond any expiry date stated by the manufacturer.

American National Standard ANSI/AAMI ST59:1999

Sterilization of health care products—Biological indicators—Part 1: General

1 Scope

This standard, ANSI/AAMI ST59, specifies general production, labeling, and performance requirements for the manufacture of biological indicators (BIs) and suspensions intended for use in the validation and monitoring of sterilization cycles.

NOTE—ANSI/AAMI ST21 and ANSI/AAMI ST19 specify the particular requirements for BIs for defined sterilization processes.

This document does not contain requirements for product directly inoculated with test organisms or recovery procedures for such inoculated product.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of ANSI/AAMI ST59. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on ANSI/AAMI ST59 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. The American National Standards Institute (ANSI) maintains a register of currently valid American National Standards.

2.1 STUMBO, CR., MURPHY, JR., AND COCHRAN, J. Nature of thermal death curves of PA 3579 and *Clostridium botulinum*. *Food Technology*, 1950, vol. 4, pp. 321–326.

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

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

2.4 ANSI/AAMI ST21:1999. *Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization.*

2.5 ANSI/AAMI ST19:1999. *Sterilization of health care products—Biological indicators—Part 3: Biological indicators for moist heat sterilization.*

2.6 U.S. FOOD AND DRUG ADMINISTRATION, Department of Health and Human Services, Part VII. 21 CFR Parts 808, 812, and 820 Medical Devices; Current Good Manufacturing Practice (CGMP); Final Rule (“Quality System Regulation”). *Federal Register*, Vol. 61, No. 195, Monday, October 7, 1996.

3 Definitions

For the purposes of this American National Standard, the following definitions apply.

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

3.2 carrier: Supporting material on which test organisms are deposited.

3.3 primary pack: System which protects the inoculated carrier from damage and contamination without preventing penetration of the sterilizing agent(s).

3.4 secondary pack: Container system in which biological indicators are packed for transport and storage.

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

3.6 test organism: Microorganism used for the manufacture of inoculated carriers.

3.7 viable test organism count: Number of viable test organisms in a unit volume of a suspension or on an inoculated carrier, estimated by growth of discrete colonies under the stated culture conditions.

3.8 inactivation: Loss of the ability of the test organisms to germinate, outgrow, and/or multiply under the specified culture conditions.

3.9 culture conditions: Manufacturer's stated combination of conditions, including the growth medium with the period and temperature of incubation, used to promote germination, outgrowth, and/or multiplication of the test organism.

3.10 recognized culture collection: International depository authority under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent and Regulation.

3.11 D-value; Decimal reduction value: Exposure required to secure inactivation of 90% of a population of test organisms under stated conditions.

3.12 survivor curve: Graphical representation of inactivation against increasing exposure to stated conditions.

3.13 process challenge device: Object that simulates for the sterilizing agent(s) the worst case conditions in the items to be sterilized.

NOTES—

1. The process challenge device is so constituted that a BI can be arranged in the place most difficult for the sterilizing agent(s) to reach.

2. The design of the process challenge device depends on the kind of goods to be sterilized and the sterilization procedure. The BI should not interfere with the function of the challenge device.

3. In some process challenge devices, an inoculated carrier may be used instead of a BI.

3.14 colony forming unit (CFU): Visible growth of microorganisms arising from a single cell or multiple cells.

3.15 self-contained biological indicator: BI presented in such a way that the primary pack, intended for incubation, contains the growth medium required for recovery.

3.16 survival-kill window: Extent of exposure to a sterilization process under defined conditions when there is a transition from all BIs showing growth (survival exposure) to all BIs showing no growth (kill exposure).

3.17 nominal population: Stated number of microorganisms.

NOTE—The actual number of microorganisms will differ from the nominal population of microorganisms as a result of the accuracy of the inoculation and recovery methods.

3.18 resistometer: Equipment designed to create defined combinations of the physicochemical variables of a sterilization process within defined limits.

4 Production, performance, and labeling requirements

4.1 Manufacturing controls and quality systems

4.1.1 All operations required by this part of ANSI/AAMI ST59 shall be controlled according to a quality system complying with the requirements of the ISO 9001, ISO 9002, and/or Quality System Regulation (QS Regulation).

4.1.2 Traceability of manufacturing components shall be maintained.

Manufacturing components should include all materials and components incorporated in or coming into direct contact with the test organism suspension, the inoculated carrier, or the biological indicator.

4.1.3 The finished product supplied by the manufacturer (suspension, inoculated carrier, or BIs) shall have no organisms, other than the test organism, present in sufficient numbers to impair the utility of the product. This shall be validated, controlled, monitored, and recorded.

4.2 Test organisms

4.2.1 Test organisms shall be of a strain suitable for handling without special containment facilities.

4.2.2 Test organisms shall be of a defined strain, lodged with a recognized culture collection, and shall be unambiguously identified by reference to the culture collection number.

4.2.3 When the strain of the test organism to be used is not already lodged with a recognized culture collection, the manufacturer shall be responsible for lodging that particular strain with a recognized culture collection.

4.2.4 The originating inoculum for each batch of test organism suspension shall be

a) traceable to the reference culture lodged with the recognized culture collection;

b) verified as to its identity and purity.

The method(s) used for the maintenance of cultures of the test organism should be designed and maintained to ensure that the cultures are protected from contamination and induced changes in their inherent properties.

Verification tests are specific for each strain of test organism and should be documented and validated by the manufacturer.

4.3 Test organism suspensions

4.3.1 The culture medium and incubation conditions used for preparation of the test organism suspension shall be defined by the manufacturer. These conditions shall consistently produce test organism suspensions that meet the performance requirements of this American National Standard and the particular performance requirements provided in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

4.3.2 The method of harvesting and subsequent treatment shall ensure that the suspension to be used in the inoculation of carriers is free from residues of the culture medium that could adversely influence the performance of the inoculated carrier or biological indicator.

This shall not be required where the manufacturer has demonstrated that residues of the culture medium do not adversely influence the performance of the inoculated carrier or BI.

4.3.3 Manufacturers of test organism suspensions and/or BIs shall maintain adequate records in order to allow traceability of BIs and test organism suspension back to the culture obtained from the culture collection.

4.3.4 If a test organism suspension is distributed for use in the preparation of inoculated carriers or the preparation of inoculated product, then each container of test organism suspension shall be accompanied by the following information:

a) the name of the test organism;

b) the name or abbreviation of the culture collection from which the test organism has been obtained and the reference number of the species;

c) the nominal volume of suspension in milliliters (or in grams, if not a suspension);

d) a unique code by which the manufacturing history can be traced;

e) the viable count in test organisms per milliliter;

f) the recommended storage conditions;

g) the expiry date or shelf life;

NOTE—Although compliance with ISO 11138-1 requires compliance with ISO 8601 (i.e. YYYY-MM-DD), it is not a requirement of this American National Standard, ST59.

h) the manufacturer's name, trademark, address, or other means of identification;

i) disposal instructions.

4.3.5 When requested by the purchaser, the manufacturer shall supply details of the resistance and performance characteristics of the suspension. These data shall be determined by a method agreed upon by the purchaser and the manufacturer.

4.3.6 The conditions for storage of suspensions of test organisms and their expiry date shall be defined by the manufacturer. These conditions shall be monitored during storage. These conditions shall maintain the test organism suspensions so that they continue to meet the performance requirements of this American National Standard and the particular performance requirements provided in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

4.3.7 The viable test organism count of the suspension shall be determined.

Where the user requires information on the growth index of the test organism, this should be determined by expressing the viable test organism count as a percentage of the total count determined microscopically.

4.3.8 The manufacturer shall specify transportation, storage and handling conditions compatible with the storage conditions specified for the suspension of test organisms (see 4.3.6).

4.4 Carrier, primary packaging, and design

4.4.1 Carriers and primary packaging shall not contain any contamination (physical, chemical, or microbial) that would adversely affect the performance of the BI.

4.4.2 The carrier and primary packaging shall not be degraded by the sterilization process with which it is intended to be used in such a way that the performance characteristics of the inoculated carrier are adversely affected.

The carrier should withstand transport in the primary and secondary pack and handling at the point of use without breakage.

The design of the carrier and/or primary pack should be such that

a) it will minimize the loss of the original inoculum of test organisms during transport and handling and during shelf life;

b) it is appropriate for use as part of a process challenge device.

4.4.3 Compliance with 4.4.2 shall be tested by observation of carrier and primary packaging exposed to extreme ranges and rates of change of chemical and physical variables of the sterilization process.

NOTE—These limits are given in the relevant subsequent parts of ANSI/AAMI ST21 and ANSI/AAMI ST19.

4.4.4 During and after the sterilization process, the carrier and primary package shall neither retain nor release any substance to such an extent that on transfer to the growth medium, under the culture conditions, there will be inhibition of the growth of low numbers of surviving test organisms.

Tests for compliance with this requirement shall be performed in accordance with annex F.

4.4.5 The manufacturer shall provide the purchaser with a statement of the maximum and minimum values of each dimension of the carrier on request.

4.5 Inoculated carriers

4.5.1 In the preparation of a batch of inoculated carriers, only one strain of a species of test organism shall be used, unless the manufacturer has demonstrated that the use of multiple strains or species does not significantly affect indicator organism resistance to the specified sterilization process.

4.5.2 Inoculated carriers shall be prepared by inoculating carriers with test organism suspension, followed by drying under controlled conditions.

4.5.3 The conditions under which inoculation is carried out shall be specified, validated, and controlled to ensure that the inoculated carrier remains free from microorganisms, other than the test organism, that may affect adversely the performance of the product as specified in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

4.5.4 The same nominal population of test organisms shall be deposited on each inoculated carrier used in the manufacture of a batch of BIs.

4.5.5 The conditions for storage of inoculated carriers and their expiry date shall be defined by the manufacturer. These conditions shall be monitored during storage. These conditions shall maintain the performance requirements of this standard and for the particular performance requirements for inoculated carriers in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

4.5.6 Where the inoculated carriers are packaged for conversion into BIs, they shall be packaged in a manner that does not adversely affect the nominal population or performance of individual inoculated carriers.

4.5.7 Each batch of inoculated carriers shall be accompanied by the following information:

a) “inoculated carriers”;

b) the name of the test organism;

c) directions for use, especially data about the medium and conditions to be used for recovery of test organisms after exposure to the sterilization process;

d) the name of the culture collection from which the test organism has been obtained and the reference number of the species;

e) the number of test organisms per inoculated carrier;

f) the batch number or unique code by which the manufacturing history can be traced;

g) data about the resistance characteristics of the inoculated carriers to the sterilization process for which they are suitable, including the test conditions and methods used to determine these characteristics;

h) the number of inoculated carriers in the secondary pack;

i) the recommended storage conditions;

j) the expiry date of the inoculated carriers;

NOTE—Although compliance with ISO 11138-1 requires compliance with ISO 8601 (i.e. YYYY-MM-DD), it is not a requirement of this American National Standard, ST59.

k) the manufacturer's name, trademark, address or other means of identification;

l) the sterilization process for which the inoculated carriers are suitable;

m) disposal instructions.

4.6 Biological indicators (BIs)

4.6.1 Biological indicators shall be prepared by packaging individual inoculated carriers in a primary pack.

4.6.2 The primary packaging shall be designed, constructed, and validated to ensure that the BI presented in the primary packaging meets the performance requirements of ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

4.6.3 The primary packaging shall be designed, constructed, and validated to ensure that when stored and transported in accordance with the manufacturer's instructions, the BI and inoculated carrier are protected from both contamination and loss of the inoculum from the carrier.

4.6.4 The conditions under which primary packaging is carried out shall be specified, validated, and controlled to ensure that the inoculated carrier remains free from microorganisms (other than the test organism) that may affect adversely the performance of the product as specified in ANSI/AAMI ST21 and ANSI/AAMI ST19.

4.6.5 The primary pack shall be validated for its intended use. Appropriate national or international standards should be used.

4.6.6 Each BI primary package shall be labeled with the following information:

a) the name of the test organism;

b) the batch number of the BI;

c) the expiry date of the BI;

NOTE—Although compliance with ISO 11138-1 requires compliance with ISO 8601 (i.e. YYYY-MM-DD), it is not a requirement of this American National Standard, ST59.

d) an indication of the sterilization process for which the BI is suitable;

e) the manufacturer's name, trademark, address or other means of identification.

4.6.7 BIs shall be packed in a secondary pack for transport and storage.

4.6.8 The secondary pack shall be labeled with the following information:

a) "Biological Indicators";

b) the information specified in 4.6.6;

c) the name of the culture collection from which the test organism has been obtained and the reference number of the species;

d) the number of test organisms on each BI as determined for the batch of inoculated carriers;

e) the number of BIs in the secondary pack;

f) the recommended storage conditions;

g) the resistance of the test organism on the inoculated carrier in its primary pack, including the test conditions and methods used to determine these characteristics;

h) directions for use, especially data about the medium and conditions to be used for recovery of test organisms after exposure to the sterilization process;

i) disposal instructions.

4.6.9 Each secondary pack shall be supplied with a copy of a certificate for that batch of BIs which shall include the following information:

a) information specified in 4.6.8;

b) data about the resistance characteristics of the indicators to the sterilization process for which the BIs are suitable for monitoring;

c) a reference to this American National Standard and any other relevant national or international standards.

4.6.10 Each secondary pack shall be supplied with written instructions for users for the handling and recovery of the BIs, and the directions shall state the following instructions:

a) BIs shall be stored under the conditions specified by the manufacturer.

b) BIs of a batch shall not be used beyond the expiry date stated by the manufacturer.

c) After exposure to the sterilizing procedure to be tested, the BIs shall be examined for recoverable test organisms within a time period specified by the manufacturer. If times other than those specified by the manufacturer are used, those times shall be validated.

d) When BIs are examined for recoverable test organisms, the methods and conditions prescribed by the manufacturer shall be applied. If alternative methods are used, those methods shall be validated.

4.7 Self-contained biological indicators

4.7.1 Self-contained biological indicators shall comply with all of the requirements of this American National Standard and with ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

4.7.2 The self-contained BI system should be sufficiently robust to withstand transport in the secondary pack as well as handling at the point of use without breakage.

The design of the self-contained BI system should be such that

a) it will minimize the loss of the original inoculum of test organisms during transport and handling;

b) it is appropriate for use as part of a process challenge device.

4.7.3 During or after the sterilization process, the materials comprising the self-contained BI system shall neither retain nor release any substance to an extent that will inhibit the growth of low numbers of surviving test organisms under culture conditions (see annex F).

5 Determination of resistance

5.1 Resistance testing requirements

5.1.1 The resistance of each batch of BIs shall be tested to demonstrate conformance with the performance requirements specified in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

5.1.2 Resistance testing (see 5.4 and 5.5) shall include determination of the number of recoverable test organisms and determination of the resistance characteristics by a combination of two or more of the following methods:

a) determination of the D-value through the construction of a survivor curve;

b) determination of the D-value through fraction negative analysis;

c) calculation of the survivor-kill window using the determined D-value, and verification of the survival-kill response characteristics.

5.1.3 The values obtained by these tests shall be within the ranges specified in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate. At least two of these values shall be stated on the labeling of the secondary pack (4.6.8) and the certificate accompanying each batch of inoculated carriers (4.5.7).

NOTE—Relevant subsequent parts of the standard (e.g., ANSI/AAMI ST21 and ANSI/AAMI ST19) may require additional determinations (e.g., a z-value for moist heat BIs).

5.2 Calculation of the survival-kill window

The survival-kill window shall be calculated by using one of the D-values determined in annex B and annex D or G along with the following formulas:

Survival exposure = not less than the D-value X ($\log_{10}$ labeled viable test organism count per carrier – 2)

Kill exposure = not more than the D-value X ($\log_{10}$ labeled viable test organism count per carrier + 4)

5.3 Determination of the number of recoverable test organisms

The number of recoverable test organisms shall be determined in accordance with annex A.

5.4 D-value determination

5.4.1 The data used in the calculation of a D-value for the biological indicators shall be determined according to annex B (i.e. construction of a survivor curve using direct enumeration) and/or annex C (fraction negative analysis or Most Probable Number (MPN) method).

5.4.2 The D-value shall be calculated in accordance with annex B and/or annex D or G.

5.4.3 Other methods of analyzing fraction negative data may be used, but relationship with the reference methods must be demonstrated.

5.5 Survival-kill response determination

The survival-kill response characteristics shall be determined and verified in accordance with annex E.

Annex A
(normative)

Determination of viable test organism count

A.1 Inoculated carriers shall be examined for recoverable test organisms in accordance with A.2 through A.4 or by an alternative method. If an alternate method is used, it shall have a known relationship with the reference method.

A.2 A minimum of four test pieces from each lot, batch, or exposure shall be used. Each test piece shall be placed into an appropriate volume of suspending menstrum. The test organisms shall be eluted from the inoculated carriers by a specified procedure (e.g., shaking with glass beads, grinding in a homogenizer, ultrasonication, or other appropriate procedure).

A.3 The suspensions shall be diluted in appropriate sterile dilution fluid to yield 30 to 300 colony-forming units (CFUs) per aliquot to be plated. Appropriate aliquots shall then be either mixed with molten tempered agar medium or spread on plates of solidified agar medium. The manufacturer of the BIs shall identify or make available a suitable recovery medium and/or the complete data for the preparation of one.

A.4 The plated samples shall be incubated at temperatures and times determined by the manufacturer.

NOTES—

1. Generally, the incubation times and temperatures are 55º C to 60º C for not less than 48 hours (h) for thermophilic organisms and 30º C to 37º C for not less than 48 h for mesophilic organisms.

2. Desiccation of growth media can adversely affect growth at elevated incubation temperatures.

A.5 After the appropriate incubation period, the numbers of CFUs on the plates shall be counted, and the mean number of recoverable test organisms per inoculated carrier shall be calculated.

Annex B
(normative)

Survivor curve method

NOTE—The ideal survivor curve is linear over the full range of inactivation. In practice, deviations from this ideal occur, but linearity must be maintained within acceptable limits. Construction of a survivor curve by direct enumeration establishes the resistance for surviving populations greater than 5×101 whereas the MPN method establishes the resistance for surviving populations below 5×100. Good correlation of the D-values obtained by the two methods can therefore be used to establish that there are no serious deviations from a linear survivor curve.

B.1 Test samples shall be subjected at graded exposures to the defined exposure conditions. The range of exposures shall be stated.

NOTE—Details of the performance requirements for exposure apparatus are given in relevant subsequent parts of ANSI/AAMI ST21 or ANSI/AAMI ST19, as appropriate.

B.2 A minimum of five exposures shall be used and shall include:

a) one exposure in which the sample is not subjected to the sterilizing agent (the sterilizing agent may be absent or replaced by a non-lethal gas);

b) reduction of the viable population to not more than 0.01% of the original inoculum with at least one exposure employed;

c) a minimum of three exposures that span the range of items (a) and (b) above.

B.3 Not fewer than four inoculated carriers shall be used for each exposure in each determination. The same number of replicates shall be utilized for each exposure.

B.4 Within 2 h after each exposure, the test samples shall be treated to remove the test organisms from the carrier, and a viable count assay shall be performed using the specified culture conditions and methods stated by the manufacturer.

B.5 Using all of the data obtained, plot the $\log_{10}$ of the surviving population against interval and determine the best fit rectilinear curve by regression analysis using the method of least squares. Survivor data points within 0.5-logarithm of the initial population shall not be included in the regression analysis. Calculate the negative reciprocal of the slope of the line obtained, which is equal to the D-value in minutes (min).

B.6 The value obtained for the correlation coefficient for the linearity of the survivor curve shall be not less than 0.8.

Annex C
(normative)

Fraction negative analysis or Most Probable Number (MPN) method for subsequent determination of D-value

NOTE—The fraction negative data are generally used to calculate the response characteristics of the test organisms to the sterilization process being used by means of the MPN method.

C.1 The samples shall be subjected at graded exposures to the defined exposure conditions with all process variables except exposure time remaining constant. No fewer than 20 replicates shall be used at each exposure. Each exposure time shall differ from the previous exposure time by a constant interval. The same number of replicates shall be used at each exposure.

NOTE—Details of the performance requirements for exposure apparatus are given in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

C.2 Within 2 h of each exposure, each inoculated carrier is transferred aseptically to a test tube containing an adequate volume of the specified sterile culture medium. The volume of medium shall be the same for each replicate. If the medium is included by the manufacturer as an integral part of the BI, the manufacturer's culturing instructions shall be followed. The manufacturer of the BIs shall identify or make available a suitable recovery medium and/or the complete data for preparation of one.

C.3 The inoculated carriers are incubated at the temperature recommended by the manufacturer. The cultures are examined for the presence of growth of the test organism after the manufacturer's recommended incubation time. Growth of the test organism can be indicated by turbidity of the broth medium, growth on the surface of the broth in the form of pellicle, sediment at the bottom of the tube (depending upon the characteristics of the test organism), or change of physical appearance of the media itself, for example pH change-induced defined transition of color.

C.4 The results are recorded as the ratio of inoculated carriers with recoverable test organisms to the total number of inoculated carriers exposed at each exposure.

NOTE—Constant exposure times are not required when using annex G. Constant sample size is not required for calculations.

Annex D
(normative)

Calculation of D-value using the Limited Spearman-Karber Procedure

D.1 Calculation of D-value

NOTE—The Limited Spearman-Karber procedure requires that successive exposures differ by a constant interval (d) and that the same number of replicates (n) are exposed at each interval.

D.1.1 The exposure interval or level (U_1, U_2....U_k) shall be chosen to cover the fraction-negative region completely. There shall be an initial exposure (U_1) to show zero sterile replicates or r = 0. The last exposure (U_k) shall have all sterile replicates or r = n. The test is valid if there are no negative units (r = 0) at the exposure preceding U_1 and all negative replicates (r = n) at the exposure subsequent to U_k, and at least two fractional response intervals (where r is greater than 0 and less than *n*) between U_1 and U_k. Calculate the mean exposure (U_{sk}) until sterility, or the Limited Spearman-Karber estimate, using the formula

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

where:

U_{sk} = mean exposure until sterility (Limited Spearman-Karber estimate)

U_k = first exposure to show all sterile replicates, $r_k = n$

i = exposure interval

d = interval between exposures (see note above)

n = number of replicate biological indicators at each exposure (see note above)

r_i = number of sterile replicates at each exposure

U_1 = longest exposure where none of the BIs are negative, r = 0

U_{k-1} = Exposure prior to U_k

r_i = Sum of sterile replicate BIs (r) at all exposures between U_1 and U_{k-1}

D.1.2 Calculate the D-value from the formula:

$$\text{D-value} = \frac{U_{sk}}{\log N_o + 0.2507}$$

where:

U_{sk} = Mean exposure until sterility from above

N_o = Average viable spore count per BI determined by total viable count

D.2 Calculation of the variance of U_{sk}, the standard deviation, and the confidence interval when using the Limited Spearman-Karber calculation.

NOTE—The Limited Spearman-Karber calculation procedure makes it possible also to estimate the variance of U_{sk}, and, in turn, calculate the standard deviation and determine the confidence interval.

D.2.1 Calculate the variance of U_{sk}, i.e. (U_{sk}) from the formula:

$$(U_{sk}) = \frac{d^2}{n^2(n-1)} \times \sum_{i=1}^{k-1} r_i\,(n-r_i)$$

where:

d = interval between exposures

n = number of replicate units at each exposure

r_i = number of sterile replicates at each exposure

$\sum_{i=1}^{k-1} r_i(n-r_i)$ = sum of values derived by multiplying r_i times $(n-r_i)$ for each exposure between U_1 and U_{k-1}

D.2.2 Calculate the standard deviation of U_{sk} ($SD_{U_{sk}}$) from the formula:

$$SD_{U_{sk}} = \sqrt{V_{U_{sk}}}$$

D.2.3 Using the lower and upper confidence limits for U_{sk} (confidence limits for U_{sk} are $U_{sk} \pm 2SD_{U_k}$; p = 0.95), calculate the lower and upper confidence limits for D as

95% lower confidence limit for D:

$$D = \frac{U_{sk} - 2\,SD(U_{sk})}{\log 10\,(N_o) + 0.2507}$$

95% upper confidence limit for D:

$$D = \frac{U_{sk} + 2\,SD(U_{sk})}{\log 10\,(N_o) + 0.2507}$$

Annex E
(normative)

Survival-kill response characteristics

NOTE—Monitoring the survival-kill response characteristics of a lot of BIs provides an additional means of assuring the consistent performance of all units within a given batch.

E.1 The D-value data calculated by either construction of a survivor curve or fraction negative analysis (see annexes B, D, and G) shall be used in the calculation of the survival and kill exposure.

E.2 No fewer than 50 replicates each shall be used to confirm the survival exposure and the kill exposure.

E.3 The survival performance is the labeled exposure that results in surviving organisms on/in each unit of the sample. The kill performance is the labeled exposure that results in kill of all organisms on/in each unit of the sample.

E.4 Survival-kill performance characteristics shall be determined in a biological indicator resistometer. Survival and kill exposures are defined as follows:

Survival exposure = not less than ($\log_{10}$ labeled viable test organism count – 2) x D-value

Kill exposure = not more than ($\log_{10}$ labeled viable test organism count population + 4) x D-value

NOTE—The number of units run per exposure will depend on both the capacity and the operating characteristics of the BI resistometer being used. It may be necessary to run several exposures at both the survival and kill exposures to test the total number of units required.

Annex F
(normative)

Determination of growth inhibition by carriers and primary packaging materials exposed to the sterilization processes

F.1 Materials

F.1.1 Suspension of test organisms of the same strain and prepared in the same manner as the organisms to be used for inoculation of carriers. The suspension shall be of known population (determined by viable count) to permit dispensing of aliquots with a population of 10 to 100 viable microorganisms.

F.1.2 Resistometer complying with the relevant subsequent part of this standard.

F.1.3 Growth medium as specified in the culture conditions.

F.1.4 Incubator set and monitored to the temperature specified in the culture conditions.

F.2 Method

F.2.1 Take a representative sample of 12 uninoculated carriers and divide into six groups of two. Prepare nine containers of growth medium.

F.2.2 Package each of the two carriers from each of three of these groups in the material used in the manufacture of the biological indicators and then expose them to the sterilization process.

F.2.3 Set the operational conditions of the resistometer to the values specified in ANSI/AAMI ST21 and ANSI/AAMI ST19, as appropriate.

F.2.4 After exposure to the process, as soon as possible but in any case within 120 min of the end of the process, unwrap the carriers and aseptically transfer them to the growth medium without subjecting them to intermediate treatment. Record the time taken to complete the transfer.

F.2.5 Place one group of two carriers in each of the three containers of growth medium which is at the incubation temperature. Incubate the growth medium at the stated temperature for 2 hours (h) to allow any inhibitory substances to desorb from the carriers. Remove the growth medium from the incubator and inoculate with a volume of the test organism suspension calculated to contain fewer than 100 viable microorganisms. Return inoculated media to the incubator and incubate for the time stated by the manufacturer for the recovery of BIs under normal conditions of use.

F.2.6 Negative control: Transfer one group of two carriers, not exposed to the process, to each of the three containers of growth medium incubated for 2 h, inoculate with fewer than 100 viable microorganisms, and incubate for the stated recovery period in the same manner as described above.

F.2.7 Positive control: Incubate three containers of growth medium, containing no carriers, for 2 h, inoculate with fewer than 100 viable microorganisms, and incubate for the stated recovery period in the same manner as described above.

F.2.8 At the end of the stated recovery period, remove all nine containers from the incubator and examine them for viable organisms in accordance with the manufacturer's instructions.

F.2.9 Report results as either "growth" or "no growth" of the test organism.

F.3 Interpretation of results

F.3.1 If "no growth" occurs in one or more of the positive controls, the test procedure shall not be regarded as valid.

NOTE—No growth in the positive control may be indicative of failure to control the population of the test organism inoculum, or of inappropriate recovery conditions (growth medium, temperature, etc.).

F.3.2 If "no growth" occurs in one or more of the negative controls, the carrier shall not be regarded as suitable for the manufacture of inoculated carriers or BIs.

NOTE—No growth in the negative control where growth was obtained in the positive control may indicate that the material of which the carrier is made is itself inhibitory to the growth of the test organism.

F.3.3 If “no growth” occurs in one or more of the three tests on carriers exposed to the process, the carrier shall be regarded as not suitable for the manufacture of inoculated carriers or BIs.

NOTE—No growth may be caused by either high levels of adsorption/absorption of sterilizing agent or by degradative changes in the material of the carrier during the process.

F.4 Determination of growth inhibition by packaging materials

Samples of the primary packaging material shall be tested in a similar manner to the carrier (i.e. following the steps given in F.1 through F.3).

The test shall be carried out with portions of the primary packaging material sufficient to provide an immersed area in the growth medium equivalent to twice the area normally in contact with the inoculated carrier, or for self-contained BIs, equivalent to the area normally in contact with the recovery medium.

F.5 Flow diagram

(See next page.)

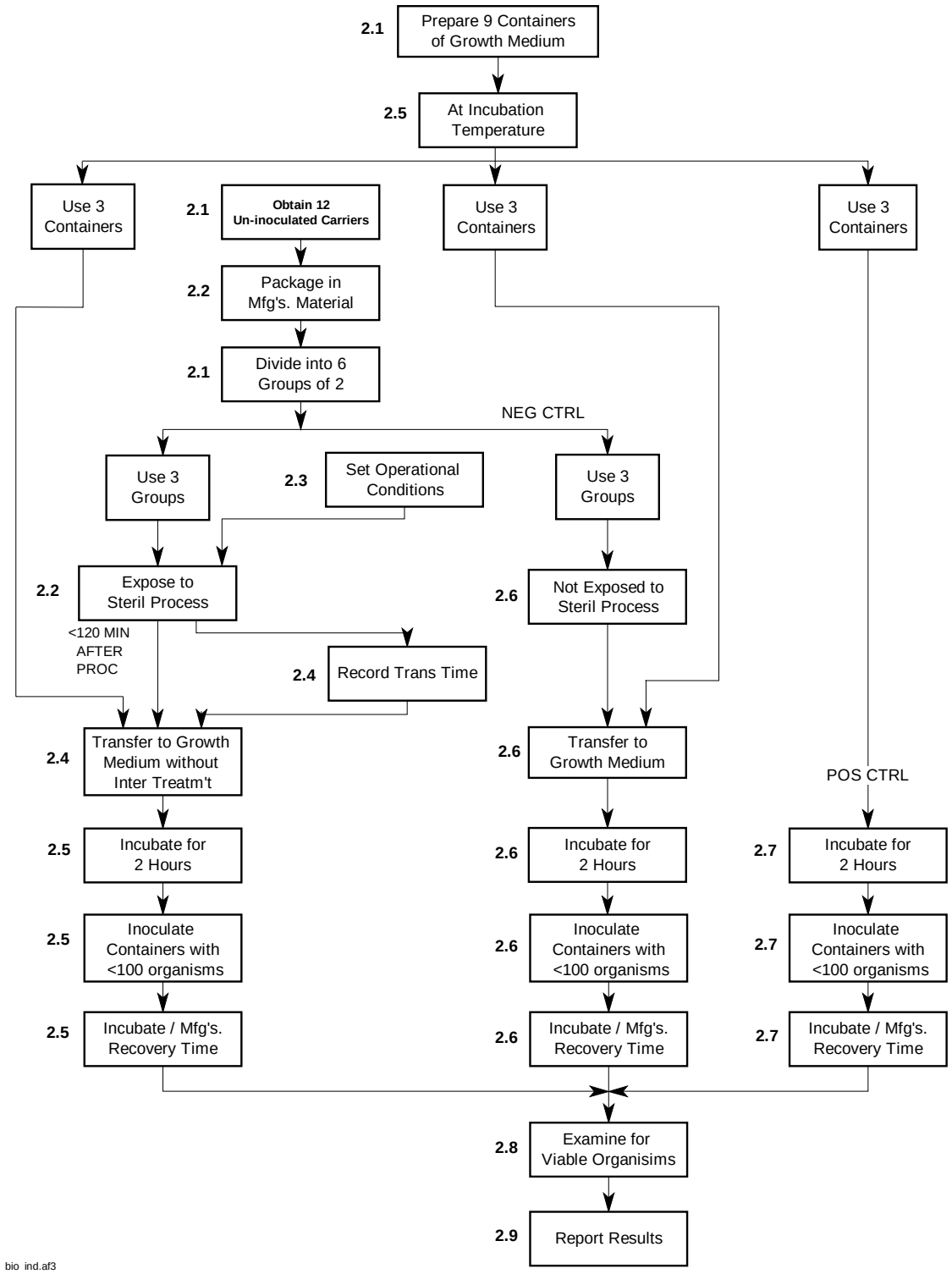

Figure F.1—Determination of growth inhibition by carriers and primary packaging materials exposed to the sterilization processes

Annex G
(normative)

Calculation of D-value using the Stumbo-Murphy-Cochran Procedure

G.1 General

The Stumbo-Murphy-Cochran method requires calculating a D-value for each exposure yielding fractional data and then, if more than one data point is available, averaging the resulting D-values to obtain the final result.

NOTE—Constant exposure times are not required for the Stumbo-Murphy-Cochran method.

G.2 Data

The following pieces of data are required to perform the Stumbo-Murphy-Cochran Procedure:

a) one or more results in the fraction-negative range, each consisting of the exposure (U), the number of units negative for growth (r), the number of replicates (n) at each exposure time;

b) the initial number of microorganisms per replicate unit (N_o)

G.3 Equation

The equation is:

$$D = \frac{U}{\log N_O - \log N_U}$$

where:

U = exposure interval

N_o = initial number of organisms per replicate carrier unit

N_u = ln $(n/r)_1$

n = total number of replicate units at exposure U

r = number of units negative for growth at U

The above equation is for the D-value determination from a single exposure time. It is recommended that an average D-value should be determined from multiple (preferably at least 3) exposure times. Also reference G.4.

G.4 Limitations

Graham (1993) has noted that if only one data point is available, the limitations associated with the Stumbo-Murphy-Cochran Procedure include the inability to determine confidence intervals and a bias of the result.

Shintani *et al.* (1995) have noted that this method may yield an invalid result if r is close to n, but that the Stumbo-Murphy-Cochran Procedure may be superior if restricted to situations where $n \geq 50$, $r \geq 1$, and $r/n < 0.9$.

G.5 References

Additional information on the Stumbo-Murphy-Cochran Procedure can be found in the following publications:

Graham, GS., and Boris, CA. Chemical and Biological Indicators. In: Morrissey, RF. (ed.). *Sterilization Technology: A Practical Guide for Manufacturers and Users of Health Care Products*. New York: Van Nostrand Reinhold, 1993, pp. 36–69.

Pflug, IJ., and Holcomb, RG. Principles of Thermal Destruction of Microorganisms. In: Block, SS. *Disinfection, Sterilization and Preservation*. 3rd ed. Philadelphia: Lea and Febiger, 1983, pp. 751–810.

Shintani, H., Tahata, T., Hatakeyama, K., Takahashi, M., Ishii, K. and Hayashi, H. Comparison of D_{10}-Value Accuracy by the Limited Spearman-Karber Procedure (LSKP), the Stumbo-Murphy-Cochran Procedure (SMCP), and the Survival-Curve Method (EN). *Biomedical Instrumentation Technology*, 1995, vol. 29, n. 2, pp. 113–124.

Stumbo, CR. *Thermobacteriology in Food Processing*. 2nd ed. Action Press: New York, 1973.

Annex H
(informative)

Background of the development of ANSI/AAMI ST59 and rationale for national deviations

H.1 Background on development of International Standards on biological indicators

In 1994 and 1995, the International Organization for Standardization (ISO) published the ISO 11138 series of standards for BIs. This series consisted of three parts:

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*

The International Standards were developed by Working Group 4, *Biological indicators* of ISO/TC 198, *Sterilization of health care products*.

H.2 Consideration of the International Standards on biological indicators for adoption as American National Standards

Following the completion of the ISO 11138 series, the AAMI Biological Indicators Working Group agreed, in the interests of international harmonization, to consider adoption of the International Standards as replacements for two existing American National Standards—ANSI/AAMI ST19:1986/(R)1994, *Biological indicators for saturated steam sterilization processes in health care facilities*, and ANSI/AAMI ST21:1986/(R)1994, *Biological indicators for ethylene oxide sterilization processes in health care facilities*. These earlier documents had been developed by the AAMI Biological Indicators Working Group under the auspices of the AAMI Sterilization Standards Committee. They were originally published in 1986 and had been reaffirmed in early 1994, pending completion of the ISO 11138 series.

In 1995, a canvas of the AAMI Sterilization Standards Committee and the AAMI Biological Indicators Working Group was undertaken. Members of the committee and the working group were asked whether the ISO standards should be considered for adoption as American National Standards without change, should be modified for U.S. adoption, or whether the U.S. should continue to maintain ANSI/AAMI ST19 and ANSI/AAMI ST21 as domestic standards.

Based on the results of the canvas and the discussion at the meeting, the AAMI Biological Indicators Working Group agreed that several modifications were required before the ISO 11138 series would be acceptable as American National Standards. These modifications were considered necessary given traditions of use of BIs in the U.S., and were consistent with positions advocated by the U.S. during the development of the ISO standards.

H.3 National deviations

Four major changes to the ISO standards were identified as necessary before the standards would be acceptable as American National Standards.

a) *The use of dual species*: Part 1 would have to be modified to allow dual-species BIs to comply with the standards.

b) *Requirements for the population log times the D-value for moist heat indicators*: The requirements for the population log times the D-value for moist heat BIs given in Part 3 would require modification.

c) *Requirements for resistometers*: The requirements for resistometers used to test BIs have been revised to reference ANSI/AAMI ST44:1992*, BIER/EO gas vessels,* and ANSI/AAMI ST45:1992*, BIER/Steam vessels*.

d) *Reference to the Stumbo-Murphy-Cochran Procedure*: An additional annex would have to be added to Part 1 to list the SMCP as an acceptable alternative reference method to the Limited Spearman-Karber Procedure.

H.4 National deviations specific to ANSI/AAMI ST59, *Sterilization of health care products—Biological indicators—Part 1: General*

H.4.1 Changes to allow dual-species biological indicators

Changes: In order to allow dual-species biological indicators to comply with ANSI/AAMI ST59, two changes to the text were required. First, the following sentence (the last sentence of the scope of ISO 11138) was deleted:

> This document does not specify requirements for biological indicators that use more than one strain or species of microorganism on a carrier.

An addition to subclause 4.5.1 was also required as follows:

> **4.5.1** In the preparation of a batch of inoculated carriers, only one strain of a species of test organism shall be used, unless the manufacturer has demonstrated that the use of multiple strains or species does not significantly affect indicator organism resistance to the specified sterilization process.

(The underlined text was added to the American National Standard and is not included in ISO 11138-1:1994.)

Rationale: Dual-species BIs are marketed and used widely in the U.S. During the development of ISO 11138-1, the U.S. position was that the use of dual-species BIs should be allowed as long as the manufacturer has demonstrated that the use of dual species does not significantly affect indicator organism resistance.

H.4.1.1 *Changes:* The "quality system" normatively referenced in this section was changed from "ISO 9002" to the "ISO 9001, ISO 9002, and/or Quality System Regulation (QS Regulation)."

Rationale: Added ISO 9001 and Code of Federal Regulations reference to QSR for U.S. manufacturers.

H.4.2 References to other American National Standards

Changes: ISO 11138-1 makes normative reference to ISO 11138-2 and ISO 11138-3. In the American National Standard ANSI/AAMI ST59, all references to the these two International Standards have been changed to specify the American National Standard versions. The citations of ISO 11138-2 and ISO 11138-3 in the Normative References section have also been replaced with references to ANSI/AAMI ST21 and ANSI/AAMI ST19, respectively.

Rationale: When adopting ISO 11138-2 and ISO 11138-3, minor but significant national deviations were incorporated into the standards. As the references to these standards in ANSI/AAMI ST59 were normative, it was necessary that the U.S. versions (ANSI/AAMI ST21 and ANSI/AAMI ST19) be cited.

H.4.3 Clarification in annex F

Change: First sentence in F.2.5 was changed from "Place one group of two carriers in each of the three containers of growth medium previously incubated at the incubation temperature" to "Place one group of two carriers in each of the three containers of growth medium which is at the incubation temperature."

Rationale: Change made to provide clarification to sentence which was ambiguous likely due to translation from an ISO standard.

H.4.4 Reference to Stumbo-Murphy-Cochran Procedure

Changes: An additional normative annex (annex G) was added to Part 1 to include the Stumbo-Murphy-Cochran Procedure as an acceptable alternative reference method to the Limited Spearman-Karber Procedure (annex D). References to the new annex were added to the body of the standard, as appropriate, and the reference to the Spearman-Karber Procedure appearing in the title of annex C was deleted.

Rationale: Earlier draft versions of ISO 11138-1 specified that D-values could be calculated from fraction negative data by either the Limited Spearman-Karber Procedure (LSKP) or by the Stumbo-Murphy-Cochran Procedure (SMCP). The description of the SMCP was deleted from later versions and the final standard specified the LSKP as the reference method, although other methods of analyzing fraction negative data were permitted if equivalence with the reference method was demonstrated. The existing American National Standard on the use of biological indicators in industrial sterilization practice (ANSI/AAMI ST34:1991) describes both the LSKP and the SMCP and permits the use of either. A recently published paper comparing the two methods has demonstrated their general equivalence (Shintani *et al.*, 1995 (see G.5)).

H.4.5 Deletion of references to irradiation or dose

Changes: The phrase “in minutes or absorbed irradiation dose” has been deleted from subclause B.5, and all related occurrences of “time” or “dose” have been deleted or replaced with “exposure” or “interval” as appropriate.

Rationale: There is no requirement for the use of BIs in ANSI/AAMI/ISO 11137 (radiation sterilization), and the Working Group would not want to inadvertently encourage the use of BIs with radiation sterilization.

H.4.6 Minor and editorial changes

H.4.6.1 *Change to the introduction:* The following paragraph from ISO 11138-1 was deleted from the introduction in the American National Standard:

> Biological indicators should always be used in combination with physical and/or chemical monitoring in demonstrating the efficacy of a sterilizing process. When a physicochemical variable of a sterilizing process is outside its specified limits, a sterilization cycle should always be regarded as unsatisfactory, irrespective of the results obtained from biological indicators.

Rationale: As the deleted paragraph is user guidance, it is not appropriate in the introduction of a standard for the manufacture of BIs.

H.4.6.2 *Change to subclause 4.1.3:* “During production” at the end of this subclause in ISO 11138-1 has been deleted in the American National Standard.

Rationale: Production is an unclear term. What is important is that the final product be validated to show that BI performance is not impaired.

H.4.6.3 *Change to subclause 4.3.4, item (g); 4.5.7, item (j); and 4.6.6, item (c):* The phrase “expressed in accordance with ISO 8601” in ISO 11138-1 has been deleted in the American National Standard, and a new note has been added to inform readers that ISO 11138-1 requires compliance with ISO 8601. The normative reference to ISO 8601 has also been deleted from the American National Standard.

Rationale: Showing dates either in the YYYY-MM-DD or the DD-MM-YYYY mode will confuse U.S. users who are used to the MM-DD-YYYY dating. As this is an American National Standard, the U.S. system is allowed.

H.4.6.4 *Change to subclause 4.3.8:* The first sentence has been revised by replacing “ensure that transport to a third party is carried out under controlled conditions” from ISO 11138-1 with “specify transportation, storage, and handling conditions” in the American National Standard.

Rationale: The obligation of the manufacturer should be to specify the shipping and handling conditions. It is impossible to “ensure that transport to a third party is carried out under controlled conditions . . .” as required in ISO 11138-1.

H.4.6.5 *Change to subclause 4.6.10 (c):* The following sentence has been added to subclause 4.6.10, item (c) in the American National Standard:

> “If times other than those stated by the manufacturer are used, those times shall be validated.”

Rationale: The time period between exposure and test for production processes may be different from times used when testing in the laboratory. Therefore, the user should have the option of validating times other than the time or times recommended by the manufacturer, and this option clarifies the intent statement in 4.6.10 (d).

H.4.6.6 *Change to subclause B.1 and B.2:* The last sentence of B.1 in ISO 11138-1 which reads “Each exposure period or dose shall differ from the previous exposure period or dose by a constant interval” has been deleted from the American National Standard. An additional item, item (c), has been added to subclause B.2:

> c) a minimum of three exposures that span the range of items (a) and (b), above.

Rationale: Constant intervals are not necessary and present some problems. The addition of item (c) to subclause 5.3 addresses the concerns that led to the requirement in ISO 11138-1 that exposure periods or intervals differ by a constant period or interval.

H.4.6.7 *Change to subclause E.4:* “Initial population” in the formulas under subclause E.4 in ISO 11138-1 has been replaced with “labeled viable test organism count” in the American National Standard to be consistent with the same formulas as they appear in subclause 5.2.

Rationale: The AAMI Biological Indicators Working Group believed that “labeled viable organism count” was intended to be used in the formulas appearing under subclause E.4.

H.4.6.8 *Addition of subclause F.5*, flow diagram.

Rationale: The flow diagram (figure F.1) was added to help the user understand the test methods defined in annex F.

H.4.7 Other changes

Other minor national deviations were necessary to improve consistency between the different parts of ISO 11138 and also to conform with U.S. spelling. This informative annex (annex H) was also added to identify the substantive differences between the ISO Standard and the American National Standard and to provide rationale for these changes.

H.5 Harmonization of ANSI/AAMI ST59 and ISO 11138-1

It is the judgment of the AAMI Sterilization Standards Committee and the AAMI Biological Indicators Working Group that ANSI/AAMI ST59 and ISO 11138-1 are sufficiently harmonized and BIs complying with the requirements of ISO 11138-1 should be in compliance with the requirements of ANSI/AAMI ST59. Because the modifications to ANSI/AAMI ST59 are permissive rather than restrictive, however, BIs complying with ANSI/AAMI ST59 might not be in compliance with ISO 11138-1.

