Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization processes
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 decision-making.

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

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

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

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Requests for interpretations of AAMI standards and recommended practices must be made in writing, to the Manager for Technical Development. An official interpretation must be approved by letter ballot of the originating committee and subsequently reviewed and approved by the AAMI Standards Board. The interpretation will become official and representation of the Association only upon exhaustion of any appeals and upon publication of notice of interpretation in the "Standards Monitor" section of the AAMI News. The Association for the Advancement of Medical Instrumentation disclaims responsibility for any characterization or explanation of a standard or recommended practice which has not been developed and communicated in accordance with this procedure and which is not published, by appropriate notice, as an official interpretation in the AAMI News.
Sterilization of health care products—
Biological indicators—Part 2:
Biological indicators for
ethylene oxide sterilization processes

Abstract: Provides specific requirements for test organisms and biological indicators intended for use in assessing the performance of sterilizers employing pure ethylene oxide gas or admixtures of the gas with diluent gases at sterilizing temperatures within the range of 20 °C to 65 °C.

Keywords: carrier, packaging, organism, resistance
AAMI Standard

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Glossary of equivalent standards

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

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

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¹ In production
² Final approval pending
Committee representation

Association for the Advancement of Medical Instrumentation
Biological Indicators Working Group

The adoption of ISO 11138-2:2006 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 Biological Indicators Working Group had the following members:

Cochairs: Gregg Mosley
Phil Schneider

Members: Richard Bancroft, Esq., Albert Browne, Ltd.
Heidi L. Betti, CST, CRST, Mercy Medical Center, Springfield, MA
Trabue D. Bryans, AppTec
Virginia C. Chamberlain, PhD, VC Chamberlain and Assoc., Palm Harbor, FL
Carlos Chavez, PhD, Abbott Laboratories
Charles Cogdill, Boston Scientific Corporation
Joseph Conaghan, MS, Alcon Laboratories
Gary Cranston, Consulting and Technical Services/PCS
Kimbrell Darnell, Bard Medical Division
Kate Davenport, Northview Biosciences
Douglas Davie, Sterilization Validation Services
Shawn Doyle, Sterilator Company, Inc.
Sylvie Dufresne, TSO3, Inc.
Dan Floyd, RM, Nelson Laboratories, Inc
James Gibson, Jr., J.M. Gibson Associates, Odessa, FL
John Gillis, PhD, SGM Biotech, Inc.
Joel R. Gorski, PhD, NAMSA
John Grillo, PhD, Hospira, Inc.
Joyce Hansen, JM Hansen & Associates
Thomas L. Hansen, Terumo Medical Corporation
Arthur C. Harris, Cook Incorporated
John L. Holland, Becton Dickinson
Charles A. Hughes, SPS Medical Supply Corporation
Danny Hutson, Cardinal Health
Lois A. Jones, MS, Cary, NC
Linda Lavelle, Johnson & Johnson
Patrick McCormick, PhD, Bausch & Lomb, Inc.
James McGowan, Jr., BS MBA, Sterile Works, Inc.
Candace McManus, DrPH, Food & Drug Administration/Center for Devices and Radiological Health
Gregg Mosely, Biotest Laboratories, Inc.
Bobby Osburn, Department of Veteran Affairs
Wendy Royalty-Hann, Raven Biological Laboratories
Terri Rymer, Baxter Healthcare Corporation
Manuel Saavedra, Jr., Kimberly-Clark Corporation
Phil Schneider, 3M Healthcare
Zenius Seliokas, Stericon, Inc.
Andrew Sharavara, Propper Manufacturing Company, Inc.
Barb Smith, Getinge USA
Gayle Strahearn, STS Division of Ethox Corporation
Nuong Van Trinh, TYCO Healthcare/Kendall
Jonathan Wilder, H&W Technology LLC

Alternates:
Solomon Alade, PhD, Alcon Laboratories, Inc.
Richard Alexander, Abbott Laboratories
Thomas Berger, PhD, Hospira, Inc.
William Boentges, BS, Cardinal Health
Greg Crego, STS Division of Ethox Corporation
Georgina Deloatch, Propper Manufacturing Company Inc.
Christophe A. Demetrius, U.S. Food and Drug Administration
Brian Drumheller, CR Bard Medical Division
Catherine Finocchiaro, Bausch & Lomb, Inc.
Douglas F. Harbrecht, Boston Scientific Corporation
Burt Kingsbury, Terumo Medical Corporation
Garrett Krushefski, SGM Biotech, Inc.
David Liu, Johnson and Johnson
Michael Mattison, Getinge USA
Richard T. O’Donnell, Steris Corporation
Timothy Ramsey, BS, Northview Biosciences
Mike Sadowski, Baxter Healthcare Corporation
Gary Socola, SPS Medical Supply Corporation
Ralph Stick, Apptec
Craig Wallace, 3M Healthcare
Julie Wheeler, NAMSA
David Woolley, BS, Nelson Laboratories, Inc.

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AAMI Sterilization Standards Committee

Cochairs: Victoria M. Hitchins, PhD
William E. Young

Members: Trabue D. Bryans, AppTec
Virginia C. Chamberlain, PhD, VC Chamberlain & Associates (Independent Expert)
Nancy Chobin, RN, CSPDM, St. Barnabas Healthcare System (Independent Expert)
Anne M. Cofiell, CRCST, FCS, International Association of Healthcare Central Service Materiel Management
Charles Cogdill, Boston Scientific Corporation
Ramona Conner, RN, MSN, CNOR, Association of Perioperative Registered Nurses
Jacqueline Daley, Association for Professionals in Infection Control and Epidemiology
Kimbrell Darnell, CR Bard
Lisa Foster, Sterigenics International
James M. Gibson, Jr., JM Gibson Associates
Barbara J. Goodman, RN, BS, CNOR (Independent Expert)
Joel R. Gorski, PhD, NAMSA
Deborah A. Havlik, Hospira Inc.
Victoria M. Hitchins, PhD, FDA/CDRH
Richard M. Johnson, MSc, BSc, Abbott Laboratories
Lois Atkinson Jones, MS (Independent Expert)
Byron J. Lambert, PhD, Guidant Corporation/Cardiac Rhythm Management
Colleen Patricia Landers, RN, Canadian Standards Association
David Liu, Johnson & Johnson
Jeff Martin, Alcon Laboratories Inc.
Patrick J. McCormick, PhD, Bausch & Lomb Inc.
Thomas K. Moore, Getinge USA
Barry F.J. Page, Barry Page Consulting (Independent Expert)
Nancy J. Rakiewicz, Ethox Corporation
Phil M. Schneider, 3M Healthcare
Michael H. Scholla, Dupont Nonwovens
Mark Seybold, Baxter Healthcare Corporation
Andrew Sharavara, Propper Manufacturing Co Inc.
Frank Sizemore, American Society for Healthcare Central Service Professionals
Gregory O. Stecklein, MS, MSM, Cardinal Health (MP&S)
William N. Thompson, TYCO Healthcare/Kendall
John W. Walker, Steris Corporation
James L. Whitby, MA, MB, FRCP, University of Western Ontario (Independent Expert)
Thelma Wilcott, Becton Dickinson & Company
Martell Kress Winters, BS, SM, Nelson Laboratories Inc.
William E. Young (Independent Expert)

Alternates:
Lloyd Brown, TYCO Healthcare/Kendall
Lina C. Bueno, Dupont Nonwovens
Craig M. Herring, Johnson & Johnson
Clark W. Houghtling, Steris Corporation
Danny Hutson, Cardinal Health (MP&S)
Jim Kaiser, Bausch & Lomb Inc.
Susan G. Klaicik, AS, BS, International Association of Healthcare Central Service Materiel Management
Joseph J. Lasich, BS, Alcon Laboratories Inc.
Chiu Lin, PhD, FDA/CDRH
Lisa N. Macdonald, Becton Dickinson & Company
Ralph Makinen, Guidant Corporation/Cardiac Rhythm Management
Mary S. Mayo, CR Bard
David Ford McGoldrick, BS, Abbott Laboratories
Jerry R. Nelson, MS, PhD, Nelson Laboratories Inc.
Jeff Peltier, Boston Scientific Corporation
Janet Prust, 3M Healthcare
Mike Sadowski, Baxter Healthcare Corporation
Ralph Stick, AppTec
Jason Voisinet, Ethox Corporation
Valerie Welter, Hospira Inc.
William T. Young, Sterigenics International

NOTE—Participation by federal agency representatives in the development of this document does not constitute endorsement by the federal government or any of its agencies.
Background of AAMI adoption of ISO 11138-2:2006

As indicated in the foreword to the main body of this document (page xi), the International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of this standard.

ISO 11138-2:2006 was developed by ISO Technical Committee 198, *Sterilization of health care products*, to fill a need for an international standard specifying the test organisms and performance requirements for biological indicators (including inoculated carriers and suspensions) used in assessing the performance of sterilization processes employing ethylene oxide gas.

U.S. participation in ISO/TC 198 is organized through the U.S. Technical Advisory Group (TAG) for ISO/TC 198, administered by the Association for the Advancement of Medical Instrumentation (AAMI) on behalf of the American National Standards Institute (ANSI). The U.S. TAG for ISO/TC 198 made considerable contributions to this standard and supports the requirements for biological indicators specified in this document.


The ISO 11138:2006 biological indicator standards series was developed as the result of the joint revision of the ISO 11138:1994-1995 series of biological indicator standards (Parts 1-3) and the EN 866:1997-2000 series of biological indicator standards (Parts 1-8). The revised ISO 11138:2006 series of standards consist of the following parts:

- ISO 11138-1, *Sterilization of health care products — Biological indicators — Part 1: General requirements*
- ISO 11138-2, *Sterilization of health care products — Biological indicators — Part 2: Biological indicators for ethylene oxide sterilization processes*
- ISO 11138-3, *Sterilization of health care products — Biological indicators — Part 3: Biological indicators for moist heat sterilization processes*
- ISO 11138-4, *Sterilization of health care products — Biological indicators — Part 4: Biological indicators for dry heat sterilization processes*
- ISO 11138-5, *Sterilization of health care products — Biological indicators — Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*

Major changes that were made to the predicate ISO and CEN series during the revision process which are incorporated into the revised ISO 11138:2006 series of standards include:

demonstrate ISO 11138:2006 compliance by complying with the provisions of ISO 11138-1:2006 even though there is no specific subpart for irradiation).


c) Inclusion of specific information pertaining to self-contained biological indicators in ISO 11138-1:2006.

d) Inclusion of a table with consolidated labeling requirements in ISO 11138-1:2006.

e) Provision for use of biological indicators deviating from the specified minimum population and/or resistance criteria providing all other requirements of ISO 11138:2006 are met and the deviation is clearly indicated in the product labeling.

f) Allowance for the calculation of D value by either the Holcomb-Spearman-Karber, Limited Holcomb-Spearman-Karber or Stumbo-Murphy-Cochran procedures as indicated in Annex D, 11138-1:2006.

g) Allowance for the use of dual species biological indicators with appropriate documentation.


i) Removal of the \( \log_{10} \) population \( \times \) D value \( \geq 10 \) requirement in ISO 11138-3:2006 (moist heat) and 11138-4:2006 (dry heat).


The primary differences between ANSI/AAMI/ISO 11138-2:2006 and ANSI/AAMI ST21:1999 are indicated in h) above as well as the changes pertaining to ANSI/AAMI/ISO 11138-1:2006, i.e., c), d), and e) above.

AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised 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 xi, this American National Standard is identical to ISO 11138-2:2006.
Foreword

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

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 11138-2 was prepared by Technical Committee ISO/TC 198, Sterilization of health care products.

This second edition cancels and replaces the first edition (ISO 11138-2:1994), which has been technically revised.

ISO 11138 consists of the following parts, under the general title Sterilization of health care products — Biological indicators:

— Part 1: General requirements
— Part 2: Biological indicators for ethylene oxide sterilization processes
— Part 3: Biological indicators for moist heat sterilization processes
— Part 4: Biological indicators for dry heat sterilization processes
— Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes
Introduction

ISO 11138-1 specifies production, labeling, test methods and performance requirements for the manufacture of biological indicators including inoculated carriers and suspensions intended for use in validation and monitoring of sterilization processes. This part of ISO 11138 gives specific requirements for those biological indicators intended for use in ethylene oxide sterilization processes.

The intent of providing requirements in the ISO 11138 series of International Standards is to provide general requirements and requirements for test methods. This series of International Standards represents the current “state-of-the-art” according to the experts representing manufacturers, users and regulatory authorities involved in developing the standard. The intent is not to promote the use of biological indicators where such use is not advised, but rather to provide common requirements for the production of those biological indicators that are known to be in use today.

Standards exist providing requirements for the validation and control of ethylene oxide sterilization (see ISO 11135).

NOTE Some countries or regions may have published other standards covering requirements for sterilization or biological indicators.

Advice on selection, use, and interpretation of results when using biological indicators can be found in ISO 14161.
Sterilization of health care products — Biological Indicators — Part 2: Biological indicators for ethylene oxide sterilization processes

1 Scope

This part of ISO 11138 provides specific requirements for test organisms, suspensions, inoculated carriers, biological indicators and test methods intended for use in assessing the performance of sterilizers and sterilization processes employing ethylene oxide gas as the sterilizing agent, either as pure ethylene oxide gas or mixtures of this gas with diluent gases, at sterilizing temperatures within the range of 29 °C to 65 °C.

NOTE 1 Requirements for validation and control of ethylene oxide sterilization processes are provided by ISO 11135.

NOTE 2 National or regional regulations could provide requirements for workplace safety.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11138-1 apply.

4 General requirements

The requirements of ISO 11138-1 apply.

5 Test organism

5.1 The test organisms shall be spores of *Bacillus atrophaeus*, *Bacillus subtilis* or other strains of microorganisms of demonstrated equivalent performance as required by this part of ISO 11138.

NOTE 1 Some strains of *Bacillus subtilis* have been reclassified as *Bacillus atrophaeus*. 
NOTE 2  *Bacillus atrophaeus* ATCC 9372, NCTC 10073, NCIMB 8058, DSM 2277, NRRL B-4418 and CIP 77.18 have been found to be suitable.

5.2 If a test organism other than *Bacillus atrophaeus* is used, the suitability of the resistance of that test organism shall be determined.

6  **Suspension**

The requirements of ISO 11138-1 apply.

7  **Carrier and primary packaging**

7.1 The suitability of the carrier and primary packaging materials for biological indicators for use in ethylene oxide sterilization processes shall be demonstrated in accordance with the requirements of ISO 11138-1:2006, 5.2 and Annex B.

7.2 The exposure conditions for establishing compliance shall be:

a) minimum exposure temperature: $\geq 55^\circ$C;

b) sterilizing agent: ethylene oxide gas at a concentration not less than 800 mg/L at $\geq 70$ % RH;

c) maximum exposure temperature: as stated by the manufacturer;

d) exposure time: $\geq 6h$.

NOTE  These conditions have been selected to represent a realistic challenge to the carrier while remaining within the practical limits of an ethylene oxide sterilization process.

8  **Inoculated carriers and biological indicators**

The requirements of ISO 11138-1 apply.

9  **Population and resistance**

9.1 The manufacturer shall state the resistance characteristics in accordance with ISO 11138-1:2006, 6.4.

9.2 The viable count shall be stated with increments $\leq 0.1 \times 10^n$ per unit (e.g. per mL of suspension, per inoculated carrier or per biological indicator).

9.3 For inoculated carriers and biological indicators, the viable count shall be $\geq 1.0 \times 10^6$.

9.4 The resistance shall be expressed as the $D$ value in minutes at 54 °C and/or 30 °C. The $D$ value of each batch/lot of biological indicators or inoculated carriers shall be stated in minutes to one decimal place at 54 °C or 30 °C, or at both temperatures.

9.5 Suspensions, inoculated carriers or biological indicators containing *Bacillus atrophaeus* spores shall have a $D$ value of not less than 2.5 min at 54 °C and/or not less than 12.5 min at 30 °C, when tested
according to the conditions in Annex A. Other microorganisms shall have \( D \) values supporting the application.

9.6 The resistance of a biological indicator may also be indicated by the term \( F_{\text{BIO}} \) value (see ISO 11138-1:2006, 3.7).

The resistance characteristics specified in this part of ISO 11138 and any other part of ISO 11138 apply to the specific test conditions stated in the other parts.

9.7 \( D \) values are determined according to methods given in Annexes C and D of ISO 11138-1:2006.

9.8 Determination of \( D \) value and survival-kill response characteristics require the use of a resistometer applying the reference resistometer process parameters (see Annex A).

9.9 The survival-kill window can be calculated using the formulae in ISO 11138-1:2006, Annex E.

NOTE This information may be of value to the user when comparing different batches from the same manufacturer.

EXAMPLE

Using the formulae in ISO 11138-1:2006, Annex E with the minimum population and minimum \( D \) value requirements specified in this part of ISO 11138, the survival-kill response characteristics are:

- at 54 °C: survival time not less than 10 min and kill time \( \leq 25 \) min;
- at 30 °C: survival time not less than 50 min and kill time \( \leq 125 \) min.
Annex A
(normative)

Method for determination of resistance to ethylene oxide sterilization

A.1 General

This method requires the use of a test apparatus referred to as a resistometer in this part of ISO 11138. The specifications of the resistometer process parameters for ethylene oxide sterilization processes are provided in ISO 18472.

Specific requirements related to the test method are provided in A.2.

A.2 Method

A.2.1 Load the samples on to suitable sample holders.

A.2.2 Preheat the resistometer chamber to the selected testing conditions (30 °C or 54 °C).

A.2.3 Place the loaded sample holders in the chamber, close the chamber and initiate the process cycle.

A.2.4 Carry out the following sequence of operations:

— Step 1: evacuate the chamber to a vacuum set point of 10 kPa ± 0.5 kPa.

— Step 2: admit sufficient water vapour to raise the relative humidity in the chamber to 60 % ± 10 %. Maintain these conditions for a period of 30 min ± 1 min. The samples should be allowed to warm to above the dew point prior to injection of water vapor to avoid the potential for condensation.

— Step 3: admit ethylene oxide to the chamber to obtain a concentration of 600 mg/L ± 30 mg/L within 60 s. For the 0 min exposure time, no ethylene oxide shall be admitted.

— Step 4: maintain these conditions for the required exposure time ± 5 s.

— Step 5: at the end of the exposure period, evacuate the chamber to 10 kPa or less within 60 s and then admit filtered air, or an inert gas (such as nitrogen) to ambient pressure.

— Step 6: repeat step 5 four additional times.

— Step 7: at the end of the above process, remove the samples from the chamber and transfer the samples to the growth medium and incubate (see ISO 11138-1:2006, Clause 7).

A.2.5 The transfer period should be documented and the same time period should be used for all tests.
A.3 Determination of resistance

Resistance characteristics shall be determined according to methods given in Annexes C, D, and E of ISO 11138-1:2006.
Bibliography
