ANSI/AAMI ST:21:1999

# Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization

American National Standard

ANSI/AAMI ST21:1999
(Revision of ANSI/AAMI ST21:1986/(R)1994)

# Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization

Developed by
**Association for the Advancement of Medical Instrumentation**

Approved 22 September 1999 by
**American National Standards Institute, Inc.**

**Abstract:** This standard 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

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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 ST21, *Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization*, details additional specific requirements for BIs intended for use with ethylene oxide sterilization. Other parts are available, including:

*Part 1: General*

*Part 3: Biological indicators for moist heat sterilization*

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

This ANSI/AAMI Standard is not intended to stand alone and can only be used effectively with ANSI/AAMI ST59.

This American National Standard contains significant national deviations from the corresponding ISO standard. All substantive national deviations are described in annex A, and a rationale for each change is provided.

Annex A 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.

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NOTE—This foreword does not contain provisions of the American National Standard, *Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization* (ANSI/AAMI ST21:1999), but it does provide information about the development and intended use of the document.

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## Introduction

In conjunction with ANSI/AAMI ST59, this standard specifies general production, labeling, and performance requirements for the manufacture of biological indicators (BIs) intended for use as monitors of ethylene oxide 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.

# Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization

## 1 Scope

This American National Standard provides specific requirements for test organisms and biological indicators (BIs) 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. Compliance with this standard necessitates compliance with ST59, *Sterilization of health care products—Biological indicators—Part 1: General.*

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of ANSI/AAMI ST21. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on ANSI/AAMI ST21 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. The American National Standards Institute maintains a register of currently valid American National Standards.

ANSI/AAMI ST59:1999, *Sterilization of health care products—Biological indicators—Part 1: General*

ANSI/AAMI ST44:1992, *BIER/EO gas vessels*

## 3 Definitions

For the purposes of this American National Standard, the definitions given in ANSI/AAMI ST59 apply.

## 4 General

The requirements of ANSI/AAMI ST59 shall apply, except as modified in the subsequent clauses of this American National Standard.

## 5 Test organisms

The test organisms shall be spores of *Bacillus subtilis* or other strains or organisms of demonstrated equivalent performance as required by this part of ANSI/AAMI ST21.

NOTES—

1. *Bacillus subtilis* NCTC (National Collection of Type Cultures [of the U.K. Central Public Health Laboratory]) 10073 and CIP (Collection of the Institut Pasteur) 7718, and *B. subtilis* ATCC (American Type Culture Collection) 9372 and NRRL (Northern Regional Research Laboratory [of the Northern Utilization Research and Development Division of the U.S. Department of Agriculture]) B 4418 have been found to be suitable.

2. If an organism other than *B. subtilis* is used, then the suitability of the resistance of the test organism chosen should be determined during process qualification.

## 6 Suspensions

Replicate determinations of the viable test organism count on the same batch of suspension shall be within ± 35% of the nominal population.

## 7 Carrier and primary packaging

For specific requirements for the carrier and primary packaging, see ANSI/AAMI ST59, subclause 4.4.

 

The test conditions used to validate the acceptability of the carrier and primary packaging materials shall be:

*Temperature*—not less than 55° C

*Relative humidity*—not less than 70%

*Ethylene oxide concentration*—not less than 800 mg/l

*Exposure time*—not less than 6 hours (h)

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 Biological indicators (BIs)

**8.1** The number of recoverable test organisms on each BI shall be controlled during manufacture to be either within ± 50% of the nominal population stated by the manufacturer or within the minimum and maximum populations stated by the manufacturer.

**8.2** Retrospective determination of the count shall be made by performing a viable test organism count under the manufacturer's stated culture conditions on a suspension of test organisms obtained by physical removal of the test organisms from the carrier through ultrasonication, shaking with glass beads, or other appropriate validated methods. Counts obtained shall be regarded as acceptable if they are within – 50% and + 300% of the stated value or, if minimum and maximum populations are stated by the manufacturer, within those stated minimum and maximum populations.

**8.3** For inoculated carriers and BIs intended for use in routine monitoring, the nominal population shall not be less than $1 \times 10^6$ stated in increments no greater than $0.1 \times 10^6$.

NOTE—Inoculated carriers and BIs intended for other purposes (e.g., qualification, validation, or other specific tests) could require other nominal populations. Other inoculum levels may be utilized for routine monitoring of industrial ethylene oxide processes, provided that level has been shown to be appropriate during process qualification.

## 9 Resistance

**9.1** Resistance performance testing shall be performed as required in ANSI/AAMI ST59, *Sterilization of health care products—Biological indicators—Part 1: General,* section 5.

**9.2** The manufacturer shall state the D-value of each batch of BIs or inoculated carriers to an accuracy of ± 0.5 minutes.

**9.3** Determination of the resistance characteristics of each batch of BIs shall be performed according to ANSI/AAMI ST44:1992, *BIER/EO gas vessels.*

**9.4** The D-values obtained by either the survivor curve method and/or by the fraction negative analysis using the Most Portable Number (MPN) procedure (see ANSI/AAMI ST59, annexes B, C, D, and G) for test organism populations on the BI shall be not less than 12.5 minutes (min) when exposed to 600 mg/l ± 30 mg/l ethylene oxide at 60% ± 10% relative humidity and 30° C ± 1° C, and/or 2.5 min if the test conditions are the same except temperature is controlled to 54° C ± 1° C.

NOTE—If an organism other than *B. subtillis* is used, then the resistance of that organism at the sterilizing conditions should be known, and the suitability of the resistance determined. This determination should be made by the user during process qualification.

## 10 Test methods

Test methods given in this standard are reference methods. When alternative method(s) are used, these shall be defined, validated, and have proven correlation with the reference method(s).

# Annex A
(informative)

# Background of the development of ANSI/AAMI ST21 and rationale for national deviations

## A.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 biological indicators (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 (WG 4), *Biological indicators* of ISO/TC 198, *Sterilization of health care products*.

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

Following completion of the ISO 11138 series, the AAMI Biological Indicators Working Group, in the interests of international harmonization, agreed to consider adoption of the International Standards as replacements for two existing American National Standards—ANSI/AAMI ST19:1986, *Biological indicators for saturated steam sterilization processes in health care facilities*, and ANSI/AAMI ST21:1986, *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, or 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.

## A.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*.

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.

## A.4 National deviations specific to ANSI/AAMI ST21, *Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization*

**A.4.1** References to other American National Standards

*Changes:* ISO 11138-2 makes normative reference to ISO 11138-1. In the American National Standard ANSI/AAMI ST21, all references to ISO 11138-1 have been changed to specify the American National Standard version. The citations of ISO 11138-1 in the normative reference section have also been replaced with references to ANSI/AAMI ST59.

*Rationale*: When adopting ISO 11138-1, significant national deviations were incorporated into the standard. As the references to this standard in ANSI/AAMI ST21 are normative, it was necessary that the U.S. versions be cited.

**A.4.2** Changes to the performance requirements for resistometers

*Changes*: The requirements for resistometers used to test ethylene oxide BIs in ISO 11138-2 have been replaced by a normative reference requiring that equipment comply with ANSI/AAMI ST44:1992, *BIER/EO gas vessels*.

*Rationale:* ISO 11138-2 provides minimum performance requirements for resistometers used to evaluate ethylene oxide sterilization BIs, which conflicted with the requirement given in the existing American National Standard for such resistometers.

When a system is being evacuated, the temperature of the system changes considerably. A vacuum level cannot be pulled to a set point within this tight tolerance as the temperature changes will alter the consistency of the actual vacuum level obtained. The tolerance given is so tight (± 0.4 kPa) that it approaches the feasible measurement level of precision pressure instrumentation, much less achievable control levels. At the rate of vacuum change prescribed, a control system cannot measure the pressure and stop the evacuation within such a tight tolerance.

**A.4.3** Minor and editorial changes

**A.4.3.1** *Change to the introduction:* The following paragraph from ISO 11138-2 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 BIs.

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

**A.4.3.2** *Change to note under clause 5:* The following sentence has been added as a new note under clause 5 in the American National Standard:

> If an organism other than *B. subtilis* is used, then the suitability of the resistance of the test organism chosen should be determined during process qualification.

*Rationale:* ISO 11138-2 specifies that the test organisms should be ". . . *Bacillus subtilis* or strains of organisms of demonstrated equivalent performance . . ." While such organisms may be appropriate for an overkill method of process qualification, they may not be appropriate for a bioburden-based process qualification. The addition to the note is a caution to ensure that the suitability of the resistance of the test organism is determined.

**A.4.3.3** *Change to clause 7:* The term "gas concentration" appearing in ISO 11138-2 has been replaced by "ethylene oxide concentration" in the listings under the second paragraph in the American National Standard.

*Rationale:* This document covers BIs intended for use with ethylene oxide sterilization, which is clearly what is intended here.

**A.4.3.4** *Change to subclause 8.2:* The words "the midpoint between" have been replaced with "are within" in the second sentence of subclause 8.2.

*Rationale:* If a manufacturer states the minimum and maximum population for a BI, then the actual population must fall within the stated minimum and maximum populations, and it is acceptable for the actual population to fall anywhere within the stated minimum and maximum populations.

**A.4.3.5** *Change to subclause 8.3:* The following sentence has been added to the end of the note in the American National Standard:

> Other inoculum levels may be utilized for routine monitoring of industrial ethylene oxide sterilization processes, provided that level has been shown to be appropriate during process qualification.

*Rationale:* While a population of $1 \times 10^6$ may be appropriate for monitoring overkill cycles, it may not be appropriate for a bioburden-based process qualification.

**A.4.3.6** *Change to clause 9:* A new subclause 9.1 was added to reference resistance performance testing to the requirements of ST59, and the subsequent subclauses of section 9 were renumbered accordingly.

*Rationale:* To clarify that the requirements of ST21 are to be used in conjunction with the requirements of ST59.

**A.4.3.7** *Change to subclause 9.4:* The following note has been added to subclause 9.4:

> NOTE—If an organism other than *B. subtillis* is used, then the resistance of that organism at the sterilizing conditions should be known and the suitability of the resistance determined. This determination should be made by the user during process qualification.

*Rationale:* This note was added to ensure that the use of BIs with resistances other than those observed for *B. subtillis* is allowed.

### A.4.4 Other changes

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

## A.5 Harmonization of ANSI/AAMI ST21 and ISO 11138-2

It is the judgment of the AAMI Sterilization Standards Committee and the AAMI Biological Indicators Working Group that ANSI/AAMI ST21 and ISO 11138-2 are sufficiently harmonized and BIs complying with the requirements of ISO 11138-2 should be in compliance with the requirements of ANSI/AAMI ST21. Because the modifications to ANSI/AAMI ST21 are permissive rather than restrictive, however, BIs complying with ANSI/AAMI ST21 might not be in compliance with ISO 11138-2.