Technical Information Report

AAMI TIR31:2003

Process challenge devices/ test packs for use in health care facilities

AAMI Association for the Advancement of Medical Instrumentation

Process challenge devices/test packs for use in health care facilities

Approved 14 March 2003 by
Association for the Advancement of Medical Instrumentation

Abstract: This technical information report provides information that will assist health care facilities in the selection and use of process challenge devices.

Keywords: biological indicator, chemical indicator, dry heat sterilization, ethylene oxide sterilization, process challenge location, steam sterilization, table-top steam sterilization, vaporized hydrogen peroxide sterilization

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

NOTE—Documents are sorted by international designation.

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

International designation	U.S. designation	Equivalency
IEC 60601-1-2:2001	ANSI/AAMI/IEC 60601-1-2:2001	Identical
IEC 60601-2-21:1994 and Amendment 1:1996	ANSI/AAMI/IEC 60601-2-21 and Amendment 1:2000 (consolidated texts)	Identical
IEC 60601-2-24:1998	ANSI/AAMI ID26:1998	Major technical variations
ISO 5840:1996	ANSI/AAMI/ISO 5840:1996	Identical
ISO 7198:1998	ANSI/AAMI/ISO 7198:1998/2001	Identical
ISO 7199:1996	ANSI/AAMI/ISO 7199:1996/(R)2002	Identical
ISO 10993-1:1997	ANSI/AAMI/ISO 10993-1:1997	Identical
ISO 10993-2:1992	ANSI/AAMI/ISO 10993-2:1993/(R)2001	Identical
ISO 10993-3:1992	ANSI/AAMI/ISO 10993-3:1993	Identical
ISO 10993-4:2002	ANSI/AAMI/ISO 10993-4:2002	Identical
ISO 10993-5:1999	ANSI/AAMI/ISO 10993-5:1999	Identical
ISO 10993-6:1994	ANSI/AAMI/ISO 10993-6:1995/(R)2001	Identical
ISO 10993-7:1995	ANSI/AAMI/ISO 10993-7:1995/(R)2001	Identical
ISO 10993-8:2000	ANSI/AAMI/ISO 10993-8:2000	Identical
ISO 10993-9:1999	ANSI/AAMI/ISO 10993-9:1999	Identical
ISO 10993-10:2002	ANSI/AAMI BE78:2002	Minor technical variations
ISO 10993-11:1993	ANSI/AAMI 10993-11:1993	Minor technical variations
ISO 10993-12:2002	ANSI/AAMI/ISO 10993-12:2002	Identical
ISO 10993-13:1998	ANSI/AAMI/ISO 10993-13:1999	Identical
ISO 10993-14:2001	ANSI/AAMI/ISO 10993-14:2001	Identical
ISO 10993-15:2000	ANSI/AAMI/ISO 10993-15:2000	Identical
ISO 10993-16:1997	ANSI/AAMI/ISO 10993-16:1997/(R)2003	Identical
ISO 10993-17:2002	ANSI/AAMI/ISO 10993-17:2002	Identical
ISO 11134:1994	ANSI/AAMI/ISO 11134:1993	Identical
ISO 11135:1994	ANSI/AAMI/ISO 11135:1994	Identical
ISO 11137:1995 and Amendment 1:2001	ANSI/AAMI/ISO 11137:1994 and A1:2002	Identical
ISO 11138-1:1994	ANSI/AAMI ST59:1999	Major technical variations
ISO 11138-2:1994	ANSI/AAMI ST21:1999	Major technical variations
ISO 11138-3:1995	ANSI/AAMI ST19:1999	Major technical variations

International designation	U.S. designation	Equivalency
ISO TS 11139:2001	ANSI/AAMI/ISO 11139:2002	Identical
ISO 11140-1:1995 and Technical Corrigendum 1:1998	ANSI/AAMI ST60:1996	Major technical variations
ISO 11607:2003	ANSI/AAMI/ISO 11607:2000	Identical
ISO 11737-1:1995	ANSI/AAMI/ISO 11737-1:1995	Identical
ISO 11737-2:1998	ANSI/AAMI/ISO 11737-2:1998	Identical
ISO TR 13409:1996	AAMI/ISO TIR 13409:1996	Identical
ISO 13485:1996	ANSI/AAMI/ISO 13485:1996	Identical
ISO 13488:1996	ANSI/AAMI/ISO 13488:1996	Identical
ISO 14155-1:2003	ANSI/AAMI/ISO 14155-1:2003	Identical
ISO 14155-2:2003	ANSI/AAMI/ISO 14155-2:2003	Identical
ISO 14160:1998	ANSI/AAMI/ISO 14160:1998	Identical
ISO 14161:2000	ANSI/AAMI/ISO 14161:2000	Identical
ISO 14937:2000	ANSI/AAMI/ISO 14937:2000	Identical
ISO 14969:1999	ANSI/AAMI/ISO 14969:1999	Identical
ISO 14971:2000	ANSI/AAMI/ISO 14971:2000	Identical
ISO 15223:2000	ANSI/AAMI/ISO 15223:2000	Identical
ISO 15223/A1:2002	ANSI/AAMI/ISO 15223:2000/A1:2001	Identical
ISO 15225:2000	ANSI/AAMI/ISO 15225:2000	Identical
ISO 15674:2001	ANSI/AAMI/ISO 15674:2001	Identical
ISO 15675:2001	ANSI/AAMI/ISO 15675:2001	Identical
ISO TS 15843:2000	ANSI/AAMI/ISO TIR15843:2000	Identical
ISO TR 15844:1998	AAMI/ISO TIR15844:1998	Identical
ISO TR 16142:1999	ANSI/AAMI/ISO TIR16142:2000	Identical
ISO 25539-1:2003	ANSI/AAMI/ISO 25539-1:2003	Identical

Committee representation

Association for the Advancement of Medical Instrumentation

Sterilization Standards Committee

This technical information report was developed by the AAMI Process Challenge Device Working Group under the auspices of the AAMI Sterilization Standards Committee. Approval of the TIR does not necessarily imply that all working group members voted for its approval.

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NOTE—Participation by federal agency representatives in the development of this technical information report does not constitute endorsement by the federal government or any of its agencies.

Foreword

This technical information report was developed by the AAMI Process Challenge Device Working Group under the auspices of the AAMI Sterilization Standards Committee. The objective is to provide technical information that will assist health care facilities in the selection and use of process challenge devices (PCDs).

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

Introduction

This AAMI technical information report (TIR) is intended to provide technical information that will assist health care facilities in the selection and use of process challenge devices (PCDs). The PCD is intended to mimic the product and packaging that is used in a sterilization process. It challenges the sterilization process by representing the worst case conditions for the sterilizing agent to penetrate.

The design of the PCD for a particular application depends on the product being sterilized and the sterilization process. Generally, the PCD is composed of a biological indicator (BI) surrounded by a barrier material that may inhibit, to some degree, the penetration of the sterilizing agent. In some cases, an integrating chemical indicator that will perform similarly to a biological indicator in a PCD may be used in the PCD.

The BI and the barrier material that encompass a PCD are exposed to a sterilization process and the BI is then cultured to determine if there are any surviving organisms. This is a means of assessing the efficacy of the sterilization procedure for inactivating microorganisms that may be present in a routine hospital load. The BI and barrier materials are selected for their appropriateness in relation to a specific sterilization process.

Routine use of a PCD is important for monitoring of sterilization processes used in health care facilities. It is an integral part of a quality control program.

The information in this PCD TIR encompasses both instructions for the user in constructing an appropriate PCD and the proper use of PCDs for each sterilization process generally used in health care facilities. Commercially prepared PCDs are available from manufacturers. This TIR also provides the user with information on selecting a commercially available PCD and questions the user may want to ask the PCD manufacturer on the proper use of their PCD.

Process challenge devices/test packs for use in health care facilities

1 Scope

This technical information report (TIR) is intended to provide technical information that will assist health care facilities in the selection and use of process challenge devices (PCDs). It is to serve as a resource that health care personnel can use when directing questions to the manufacturer about the suitability, effectiveness, and safety of a specific PCD. Currently, there are no standards that evaluate the performance of these medical devices.

1.1 Inclusions

This TIR covers user-assembled PCDs and pre-assembled commercial PCDs for sterilization processes that have been cleared for marketing by the U.S. Food and Drug Administration (FDA).

1.2 Exclusions

Similar products used in liquid chemical sterilization processes are excluded from the scope of this TIR.

User-assembled and pre-assembled test packs used to conduct the Bowie-Dick test for air removal/steam penetration in a prevacuum steam sterilization process are excluded from the scope of this TIR. The Bowie-Dick test pack is included in annex B for reference.

2 Normative references

The following normative documents contain provisions that, through reference in this text, constitute provisions of this technical information report. For dated references, subsequent amendments to or revisions of any of these publications do not apply. However, parties to agreements based on this technical information report are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. The Association for the Advancement of Medical Instrumentation maintains a register of currently valid International Standards.

2.1 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Dry heat (heated air) sterilizers.* ANSI/AAMI ST50:1995. Arlington (VA): AAMI, 1995. American National Standard.

2.2 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Ethylene oxide sterilization in health care facilities: Safety and effectiveness.* ANSI/AAMI ST41:1999. Arlington (VA): AAMI, 1999. American National Standard.

2.3 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Flash sterilization: Steam sterilization of patient care items for immediate use.* ANSI/AAMI ST37:1996. Arlington (VA): AAMI, 1996. American National Standard.

2.4 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Steam sterilization and sterility assurance in health care facilities.* ANSI/AAMI ST46:2002. Arlington (VA): AAMI, 2002. American National Standard.

2.5 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Guidelines for the selection and use of reusable rigid container systems for ethylene oxide sterilization and steam sterilization in health care facilities.* ANSI/AAMI ST33:1996. Arlington (VA): AAMI, 1996. American National Standard.

2.6 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Steam sterilization and sterility assurance using table-top sterilizers in office-based, ambulatory-care medical, surgical, and dental facilities.* ANSI/AAMI ST42:1998. Arlington (VA): AAMI, 1998. American National Standard.

2.7 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Table-top dry heat (heated air) sterilization and sterility assurance in dental and medical facilities.* ANSI/AAMI ST40:1992/(R)1998. Arlington (VA): AAMI, 1998. American National Standard.

2.8 ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION. *Table-top steam sterilizers.* ANSI/AAMI ST55:1997. Arlington (VA): AAMI, 1997. American National Standard.

2.9 ASSOCIATION OF PERIOPERATIVE REGISTERED NURSES. *Standards, recommended practices, and guidelines.* Denver (CO): AORN, 2003.

2.10 PERKINS JJ. *Principles and methods of sterilization in health sciences.* 2ed. Springfield (IL): Charles C Thomas, 1969, pp. 205–206.

3 Definitions and abbreviations

For the purposes of this AAMI technical information report, the following definitions and abbreviations apply.

3.1 biological indicator (BI): Microbiological test system providing a defined resistance to a specified sterilization process. [ANSI/AAMI/ISO TIR 11139:2002]

3.2 Bowie-Dick test: Diagnostic test of a dynamic-air-removal steam sterilizer's ability to remove air from the chamber and prevent air entrainment.

3.3 challenge test pack: Pack used in qualification, installation, and routine quality assurance testing of hospital sterilizers.

3.4 chemical indicator (CI): System that reveals a change in one or more predefined process variables based on a chemical or physical change resulting from exposure to a process. [ANSI/AAMI/ISO TIR 11139:2002]

3.5 *D* value, D_{10} value: Time or radiation dose required to achieve inactivation of 90 % of a population of the test microorganism under stated exposure conditions. [ANSI/AAMI/ISO TIR 11139:2002]

3.6 entrainment: Collecting or transporting of solid particles or a second fluid or vapor by the flow of the primary fluid or vapor at high velocity.

3.7 heat sink: Heat absorbent materials; a mass that readily absorbs heat.

3.8 process challenge device (PCD): Item designed to simulate product to be sterilized and to constitute a defined challenge to the sterilization process, and used to assess the effective performance of the process. [ANSI/AAMI/ISO TIR 11139:2002]

3.9 process challenge location (PCL): Site in the sterilizer which simulates worst-case conditions as they are given for sterilizing agents in the goods to be sterilized.

3.10 routine test pack: Pack used for routine monitoring of hospital sterilizers.

3.11 validation: Documented procedure for obtaining, recording, and interpreting the results required to establish that a process will consistently yield product complying with predetermined specifications. [ANSI/AAMI/ISO TIR 11139:2002]

3.12 z value: Number of degrees of temperature required for a 1-logarithm change in the *D* value.

4 Historical perspective

The design of a PCD depends on the material and construction of the item to be sterilized and the sterilization method and process. Typically, a biological indicator is included in the PCD and the PCD is placed in the location deemed to be the most difficult for the sterilizing agent to penetrate. In some cases, an indicator equivalent to a biological indicator (e.g., a chemical integrator) can be used. The indicator should not interfere with the function of the PCD.

Early steam sterilization practitioners considered proper sterilization of surgical supplies in hospitals an art rather than a science. The skill of the individual operator in manipulating the valves by hand and reading the gauges of early pressure-type steam sterilizers greatly determined the outcome of the process. Even then, it was recognized that a grave need existed for a dependable type of sterilization detector. Such a control should indicate whether an adequate temperature had been reached in the load during the period of exposure to the steam.

It was obvious to the practitioners that any sterilizer that has a controlling mechanism which measures the temperature at the coolest point within the chamber overcomes the need for any other temperature indicator. Evidence of performance was accepted when temperature-controlled sterilizers were equipped with recording thermometers. The bulb of a recording thermometer was inserted in the discharge piping system from the sterilization chamber so that an exact record of the temperature in the coolest part of the chamber was obtained. Examination of the chart record of a mercury-type indicating thermometer was deemed to be an accurate

representation of how long the thermal performance had continued after the minimum permissible sterilization temperature was reached. Such a chart was a permanent record of the cycle performance.

An early device used for determining that a given temperature had been obtained was a Diack™ indicator. The Diack indicator consisted of a light brown chemical substance in tablet form contained within a hermetically sealed glass tube. It was known that the tablet would melt or fuse when subjected to a temperature of 245 °F (118 °C) for 20 to 30 minutes or when subjected to 250 °F (121 °C) for 5 to 8 minutes. At higher temperatures, above 250 °F, the time required to fuse was reduced quite rapidly. One of the important features of this device was that it had attached to it a strong thread by which the device could be drawn out from the center of a dense fabric pack for observation after sterilizing without contaminating the pack. For successful sterilization, the tablet turned a bright carmine color, but the distinctive change or indication was the melted or fused condition of the tablet.

Another device that was used, particularly to evaluate steam penetration of large, heavy, dense fabric packs, was the self-registering or lag thermometer. This type of maximum value, self-registering thermometer includes a time-lag factor, supposedly 10 or 15 minutes. Upon removal from the sterilizer, when the lag thermometer showed a temperature of 250 °F (121 °C), it was supposed to indicate that that temperature had been maintained for the lag period. Lag thermometers were expensive, easily damaged, and rather bulky—so bulky that it was difficult to use them within the center of packs and remove them without disturbing the wrapper or contaminating the pack.

For many years, these devices were adequate for the gravity displacement-type steam sterilizers. With the advent of prevacuum steam sterilizers, there came a need to assess the ability of the vacuum system to remove air and allow steam to penetrate porous loads. Of particular concern was a practical means of determining the success of the process when large, dense fabric packs (the amount of air present in woven textiles was thought to be the greatest challenge to process in a porous load) were steam sterilized. Since the fabric load itself was thought to be the most resistant to the air removal/steam penetration challenge, that load became the basis for subsequent challenges to the process.

A user-assembled challenge test pack for monitoring both air removal and steam penetration is described in ANSI/AAMI ST46. This challenge pack consists of 16 folded and stacked absorbent towels with one or more BIs (and optional chemical indicators) placed in the geometric center of the pack. This steam challenge pack is used for both empty chamber sterilizer testing after installation, relocation, sterilization failures, and major repairs, as well as for routine testing in a fully loaded chamber.

With the introduction of ethylene oxide (EO) sterilization, the hospital practitioner saw the need for a test pack that would challenge the process conditions in the EO sterilizer chamber. Based on round-robin studies, AAMI identified a user-assembled reference BI challenge test pack (ANSI/AAMI ST41). The EO challenge pack consists of two BIs inside of two disposable syringes, a plastic item, and a rubber item (EO absorbents) that are all placed in the center of four folded and stacked towels. The pack is then wrapped and taped. The EO challenge pack represents a greater challenge to the sterilizer than a typical load. It is used for qualification by sterilizer manufacturers, installation testing, and after major repair. An EO test pack for routine monitoring is also described in ANSI/AAMI ST41. The routine EO test pack uses one BI inside of a disposable syringe and a CI, both placed in the folds of a single absorbent towel. The towel is then inserted into a peel pouch. The routine EO test pack represents a typical sterilizer load and is used with a fully loaded chamber.

When manufacturers began introducing the commercial, pre-assembled, disposable test packs, they used these reference challenge test packs as the “gold standard” to demonstrate equivalency in performance in their premarket notification (510(k)) submissions to the FDA. At about the same time, the descriptions of the BI challenge test packs in the AAMI documents were updated to include the term “or equivalent” to provide for the use of disposable test packs.

5 Selection and use of process challenge devices/test packs

A PCD used to assess the effective performance of a sterilization process must create a challenge to the sterilization process that is equal to or greater than the most difficult item routinely processed. A PCD may be a user-assembled or a commercial, pre-assembled PCD. For a commercial PCD intended for use in health care settings, the manufacturer is required to submit a premarket notification. The 510(k) should show results comparing the performance of the PCD to the AAMI gold standard reference BI challenge or test pack or another legally-marketed test pack of a challenge greater than the BI itself. If there is no gold standard to be referenced for new technology sterilization processes, the same gold standard performance criteria used for the traditional processes should be used for the new technology sterilization. The manufacturer should demonstrate that the test pack to be used in these processes is able to induce an increase in the effective or observed *D* value of the biological indicator placed inside the test pack. The FDA does not perform testing; it reviews the testing data as submitted by the manufacturer. A more thorough discussion of the FDA’s involvement with these medical devices is found in annex A.

The health care user must choose a commercially prepared PCD that has received a 510(k) clearance for the specific sterilization process for which it will be used (i.e., steam, EO, dry heat) or a user-assembled test pack. The

PCD manufacturer's instructions for use should supply information about the appropriate sterilization cycles (i.e., times and temperatures, etc.) for using the PCD, what type of load content challenge the PCD represents, incubation requirements for the biological indicator, storage, and shelf life. For example, a PCD designed for a 270 °F (132 °C), 4 minute dynamic-air-removal steam sterilization cycle is too resistant for use in a 270 °F (132 °C), 4 minute gravity steam sterilization cycle. A PCD designed to represent a fabric pack challenge may not be appropriate if the load contains rigid containers. The user-assembled test pack should be appropriate for the sterilization process, the cycle parameters for which it will be used, and the load contents.

Using rigid containers for the packaging of medical devices to be sterilized adds complexity to sterilization process sterility monitoring. Before purchasing any rigid sterilization container system, the user should gather information from the container manufacturer as to the application of the container system to the type of sterilization process and the types of goods processed. A prepurchase evaluation is recommended as described in ANSI/AAMI ST33:1996. ANSI/AAMI ST33:1996, 7.7.1, states that a BI challenge test pack should be used for routine monitoring of steam-sterilized loads consisting of container systems and that a routine EO test pack should be used for routine monitoring of EO-sterilized loads consisting of container systems. The AAMI Process Challenge Device Working Group, however, recommends that loads containing rigid container systems be monitored routinely with a PCD consisting of a rigid container with biological and chemical indicators placed in the most challenging location in the container as determined during the prepurchase evaluation. A commercially available PCD will provide a challenge equal to or greater than the PCD it is intended to replace. The commercially available rigid sterilization container, intended for use in health care settings, is a medical device requiring FDA premarket clearance before it can be marketed.

When selecting a PCD, the health care user should ask the manufacturer the following questions:

— Is this PCD appropriate for the sterilization process being used?

— Is this PCD appropriate for the load contents and sterilization cycle being used?

— Has the manufacturer demonstrated the PCD's equivalency to the appropriate reference challenge test pack for the specific sterilization process and cycle being used, if applicable?

— Can this PCD be used for both sterilizer efficacy testing and routine monitoring, or can it be used only for routine monitoring?

— Does this PCD have a specific shelf life?

— How will the PCD be labeled when it is delivered (date of manufacture, guidelines for use)? Is test data available?

— Are there any specific storage requirements for this PCD?

— Do you provide documentation on the biological and chemical indicators used in your PCD?

— Do you provide a troubleshooting process to follow should the PCD indicate a failure?

6 Process challenge devices

6.1 PCDs for steam sterilization

6.1.1 Introduction

The first edition of the AAMI standard, *Good hospital practice: Steam sterilization and sterility assurance* (AAMI ST1:1980), recommended the use of a heterogeneous challenge test pack consisting of three muslin surgical gowns, 12 huck or absorbent surgical towels, 30 gauze sponges, five lap sponges, and one muslin surgical drape, all sequentially wrapped with two muslin wrappers. A BI that had been properly validated for the cycle to be used is placed inside the test pack. The test pack was recommended to be approximately $12 \times 12 \times 20$ inches in size and to weigh 10 to 12 pounds, for a resulting pack density of 7.2 pounds per cubic foot. The pack specifications were based on John J. Perkins' work to restrict the size and density of processed packs so that standard sterilization cycle parameters would have an adequate margin of safety (Perkins, 1969). This pack was adopted by organizations (AORN, 1982; General Services Administration, 1975) and individual health care facilities and became a hospital standard for biological indicator monitoring of steam sterilization process.

In the years following adoption of the $12 \times 12 \times 20$ inch test pack, comments were raised concerning difficulties in obtaining items to make up the pack, the placement of biological indicators within the pack, the appropriateness of the muslin wrapper, and the rationale for the pack contents. The Hospital Practices Working Group of the AAMI Steam Sterilization Subcommittee formed a task force to investigate these issues. The results of a survey of hospital personnel revealed a need for a simpler steam biological indicator test pack with more readily available contents. Respondents to the survey recommended that: (1) the new pack consist of materials whose properties could be

specified so that critical parameters affecting steam penetration and air removal were controlled; (2) rationale and documentation be developed to specify biological indicator placement within the pack; and (3) the pack exhibit performance characteristics essentially equivalent to the current test pack. A new AAMI 16-towel BI test pack was created that exhibited all of these characteristics.

6.1.2 Description

A PCD for a steam sterilization process is a test pack that contains a biological indicator consisting of spores of *Bacillus stearothermophilus* (now called *Geobacillus stearothermophilus*) and/or a chemical indicator (ANSI/AAMI ST46:2002, 7.4.2.3, 7.4.3, 7.5). The test pack should provide a challenge to the sterilization process that is representative of the most difficult to sterilize item in the load being processed.

The AAMI challenge and routine BI test packs prepared by the health care facility are intentionally composed of materials that are readily available to both manufacturers and health care facilities. The materials create a controlled challenge to steam penetration and air removal.

The AAMI 16-towel BI test pack became the gold standard or reference BI test pack for challenging steam sterilization processes in 1988. In 1993, AAMI recommended the use of this user-assembled 16-towel BI test pack or a commercially available disposable test pack shown to be equivalent in scientific experiments (ANSI/AAMI ST46:1993, 7.6.1). This test pack is also described in ANSI/AAMI ST46:2002, 7.5.2.

The AAMI 16-towel BI test pack is not an appropriate PCD for the flash cycle. In the flash cycle, as defined in the introduction of *Flash sterilization: Steam sterilization of patient care items for immediate use* (ANSI/AAMI ST37:1996), a PCD should consist of one or more BIs containing spores of *Bacillus stearothermophilus* (now called *Geobacillus stearothermophilus*) and a chemical indicator placed in the tray configuration to be tested. Each type of tray configuration (e.g., open surgical tray, single-wrapped surgical tray, protective organizing case, rigid sterilization container) and each type of cycle (e.g., gravity-displacement, prevacuum, steam-flush pressure-pulse, flash cycle with single wrapper) in routine use for flash sterilization should be tested separately with a PCD representing those parameters (ANSI/AAMI ST37:1996, 7.6.3).

6.1.3 Reference test packs—AAMI challenge and routine test pack

See *Steam sterilization and sterility assurance in health care facilities*, ANSI/AAMI ST46:2002, 7.5.2 and annex B, for how to prepare the AAMI 16-towel BI test pack that is used as a challenge and routine test pack for steam sterilization cycles.

See *Flash Sterilization: Steam sterilization of patient care items for immediate use*, ANSI/AAMI ST37:1996, 7.6.2 and 7.7.3.1, for how to prepare a BI test tray.

6.1.4 Types of process challenge devices

6.1.4.1 Commercial process challenge devices

Although health care facilities were provided detailed information from AAMI on how to prepare PCDs containing a BI and CI using materials available, the preparation of these packs was time consuming and pack performance varied depending on how the packs were prepared and the source of materials that made up the packs. This led to the development and availability of several commercial, pre-assembled PCDs that include a BI and CI or allow the user to add their own BI and CI. These test packs are designed to create a challenge to the steam sterilization process equivalent to the AAMI 16-towel BI reference test pack. Users of these commercial, pre-assembled PCDs should review the performance data and instructions for use as supplied by the manufacturer prior to their use. At this time, there are no commercial, pre-assembled PCDs for use in the flash sterilization process.

6.1.4.2 Health care facility-prepared process challenge devices

A health care facility-prepared PCD should create a greater challenge to the sterilization process than the load itself. For a load containing packaged items, for example, a BI in just a peel pouch or instrument tray will not create a greater challenge to the sterilization process than a fabric pack in the load itself. So, health care facility-prepared PCDs should be similar in challenge to the AAMI challenge and routine BI test packs as described in 6.1.3.

6.1.5 Recommended practices—Application and frequency of use

6.1.5.1 AAMI challenge test pack

The user-assembled AAMI 16-towel BI test pack or a commercially available disposable test pack shown to be equivalent in scientific experiments should be used during initial installation testing and after relocation, sterilizer malfunction, sterilization process failure, and any major repairs of the sterilizer, and for routine, periodic quality assurance testing of representative samples of actual products being sterilized (ANSI/AAMI ST46:2002, 7.5.1 and 7.8; see 7.5 and 7.8 for instructions on how to perform the various levels of testing).

For flash sterilization cycles, a health care facility-prepared BI test tray should be used during initial installation testing of the steam sterilizer (ANSI/AAMI ST37:1996, 7.7.2 and 7.7.3.1) and for product testing (ANSI/AAMI ST37:1996, 7.9). A BI test tray should also be used after relocation, sterilizer malfunction, sterilization process failure, and any major repairs of the sterilizer. See ANSI/AAMI ST37:1996, 7.7 and 7.9 for instructions on how to perform the various levels of testing.

6.1.5.2 AAMI routine test pack

The user-assembled AAMI 16-towel BI test pack or a commercially available test pack should be used routinely in sterilization loads at least weekly, but preferably every day that the sterilizer is in use (AAMI/ANSI ST46:2002, 7.4.3.3). If a sterilizer is designed to be used for multiple types of cycles (gravity-displacement, dynamic-air-removal, flash), each sterilization cycle type should be tested. See AAMI/ANSI ST46:2002, 7.5.4 for how to perform this routine testing. Each load containing implantable devices should be monitored and, whenever possible, quarantined until the results of the BI testing are available (AAMI/ANSI ST46:2002, 7.4.3.3).

For flash sterilization, a health care facility-prepared PCD with a BI and CI should be used to check each sterilizer at least once a week, preferably daily (ANSI/AAMI ST37:1996, 7.6.3). Each type of tray configuration (e.g., open surgical tray, single-wrapped surgical tray, protective organizing case, rigid sterilization container) and each type of cycle (e.g., gravity-displacement, prevacuum, steam-flush pressure-pulse, flash cycle with single wrapper) in routine use for flash sterilization should be tested separately with a PCD (ANSI/AAMI ST37:1996, 7.6.3). Any load containing implantable devices shall be biologically monitored (ANSI/AAMI ST37:1996, 7.6.3).

6.2 PCDs for ethylene oxide sterilization

6.2.1 Introduction

With the introduction of EO sterilization, the hospital practitioner saw the need for an easy-to-assemble test pack. This PCD would need to challenge all of the critical parameters of the EO process: EO concentration, humidity, time, and temperature. Since manufacturers of EO sterilizers did not provide a standardized PCD as was common for steam sterilization processes, AAMI originally recommended the challenge BI test pack and routine BI test pack in *Good hospital practice: Performance evaluation of ethylene oxide sterilizers—Ethylene oxide test packs* (AAMI, 1985). These packs were designed based on the scientific experience and professional judgment of the members of the AAMI Ethylene Oxide Sterilization Hospital Practices Working Group.

During the revision of the 1985 document, AAMI conducted a round-robin study to evaluate the resistance of the routine BI test pack, and the result was the continued recommendation of this pack, which was subsequently incorporated into ANSI/AAMI ST41:1999. The resistance of the challenge BI test pack has not been qualified, but because of its make-up, it offers substantially more resistance than the routine test pack.

6.2.2 Description

A PCD for an EO sterilization process is a test pack containing a biological indicator(s) consisting of spores of *Bacillus subtilis* var. *niger* (now called *Bacillus atophaeus*) and a chemical indicator (ANSI/AAMI ST41:1999,7.6.1, 7.7.2). The test pack should provide a challenge to the sterilization process that is representative of the most difficult to sterilize item in the load being processed.

The AAMI-recommended challenge and routine test packs prepared by the health care facility are intentionally composed of materials that are readily available to both manufacturers and health care facilities. This includes surgical towels, which would not normally be EO sterilized but serve as heat sinks and EO and moisture absorbents. The disposable syringe, which would normally not be reused, acts as a heat sink and restricts EO diffusion. The rubber and plastic acts as an EO absorbent.

The AAMI challenge test pack has become the gold standard or reference BI test pack for challenge testing. The AAMI EO routine BI test pack has become the gold standard or reference BI test pack for routine testing of EO sterilization process.

6.2.3 Reference test packs

6.2.3.1 AAMI challenge test pack

See *Ethylene oxide sterilization in health care facilities: Safety and effectiveness*, ANSI/AAMI ST41:1999, 7.6.1 for how to prepare an AAMI challenge BI test pack.

6.2.3.2 AAMI routine test pack

See *Ethylene oxide sterilization in health care facilities: Safety and effectiveness*, ANSI/AAMI ST41:1999, 7.7.2 for how to prepare an AAMI routine BI test pack.

6.2.4 Types of process challenge devices

6.2.4.1 Commercial process challenge devices

Although health care facilities were provided detailed information from AAMI on how to prepare PCDs containing a BI and CI using materials available, the preparation of these packs was time consuming and pack performance varied depending on how the packs were prepared and the source of materials that made up the packs. This led to the development and availability of several commercial, pre-assembled PCDs that include a BI and/or a CI or allow the user to add a BI and CI. These test packs are designed to create a challenge to the EO sterilization process equivalent to the AAMI routine BI test pack. Users of these commercial, pre-assembled PCDs should review the performance data and instructions for use as supplied by the manufacturer prior to their use.

6.2.4.2 Health care facility-prepared process challenge devices

A health care facility-prepared PCD should create a greater challenge to the sterilization process than the load itself. For example, a BI in just a peel pouch will not create a greater challenge to the sterilization process than the load itself. So, health care facility-prepared PCDs should be similar to the AAMI challenge and routine BI test packs as described in ANSI/AAMI ST41:1999, 7.6.1 and 7.7.2.

6.2.5 Recommended practices—Application and frequency of use

6.2.5.1 AAMI challenge test pack

The user-assembled AAMI challenge BI test pack or a commercially available test pack for biological monitoring that has been validated against the AAMI challenge test pack should be used for qualification testing by sterilizer manufacturers (ANSI/AAMI ST41:1999, 7.6.1 and 7.6.2) for initial installation testing (ANSI/AAMI ST41:1999, 7.6.3), for periodic quality assurance testing (ANSI/AAMI ST41:1999, 7.6.4) and after sterilization process failure (ANSI/AAMI ST41:1999, 7.7.6). See appropriate sections for instructions on how to perform the various levels of testing.

6.2.5.2 AAMI routine test pack

The user-assembled AAMI routine BI test pack or an equivalent commercial disposable test pack should be used in each sterilization cycle (ANSI/AAMI ST41:1999, 7.7.1). Each load containing implantable devices should be monitored and, whenever possible, quarantined until the results of the biological indicator testing are available (ANSI/AAMI ST41:1999, 7.5.3). See ANSI/AAMI ST41:1999, 7.7 through 7.8, for instructions on how to perform this routine testing.

6.3 PCDs for table-top steam sterilization

6.3.1 Introduction

Larger hospital sterilizers (volume greater than two cubic feet) are routinely tested using the AAMI 16-towel BI test pack or a commercial, pre-assembled test pack of equivalent performance. PCDs for sterilizer manufacturers to use for performance and design qualification of table-top steam sterilizers intended for use in health care facilities are described in ANSI/AAMI ST55:1997, but these test packs were not intended for use by health care facilities. So, there is no universally accepted reference standard PCD for table-top steam sterilizers. It is recommended that a representative package or tray that is to be routinely processed through the sterilizer be placed in the most difficult to sterilize location and be used as the PCD for challenge and routine monitoring (ANSI/AAMI ST42:1998).

6.3.2 Description

A PCD for a table-top steam sterilization process should contain a biological indicator consisting of spores of *Bacillus stearothermophilus* (now called *Geobacillus stearothermophilus*) and a chemical indicator. The test pack should provide a challenge to the sterilization process that is representative of the most difficult to sterilize item in the load being processed (ANSI/AAMI ST42:1998, 7.6.2).

6.3.3 Reference test packs

Reference test packs for manufacturers to use in the performance and design qualification of table-top steam sterilizers intended for use in health care facilities are described in ANSI/AAMI ST55:1997. However, these test packs were not intended for use by the health care facility for routine monitoring. So, there are no reference test packs. See ANSI/AAMI ST42:1998, 7.6.2 for how to select a test pack for monitoring table-top steam sterilizers.

6.3.4 Types of process challenge devices

6.3.4.1 Commercial process challenge devices

There are no commercial PCDs on the market for table-top steam sterilizers.

6.3.4.2 Health care facility-prepared process challenge devices

A health care facility-prepared PCD for table-top steam sterilizers should create a greater challenge to the sterilization process than does the load itself. The PCD should include a BI and CI. The PCD should be a package or tray that is representative of the same type of package or tray routinely processed through the sterilizer and is considered the most difficult to sterilize. Characteristics that should be considered when preparing a PCD include multiple layers of dressing materials, large metal masses, and mixed packs incorporating both. Table 1 lists examples of PCDs for gravity-displacement steam table-top sterilizers based on load contents and cycle times and temperatures.

Table 1—PCDs for gravity-displacement steam table-top sterilizers

Load	Temperature/time	Test pack
Unwrapped instruments on a tray, glassware	270 °F (132 °C)/ ≥ 3 minutes	Place BI and CI in an unwrapped instrument tray or in glassware
Wrapped trays of instruments, instruments in peel pouches	270 °F (132 °C)/ ≥ 10 minutes	Place BI and CI in a wrapped tray or peel pouch (include porous items, if appropriate)
Packs, wrapped	250 °F (121 °C)/ ≥ 30 minutes	Place BI and CI in a wrapped pack that is representative of the load (include porous items, if appropriate)

6.3.5 Recommended practices—Application and frequency of use

The user-assembled PCD for table-top sterilizers should be used for installation testing and after any major repairs (ANSI/AAMI ST42:1998, 7.6.1.1), for periodic monitoring of all types of packages and trays processed (ANSI/AAMI ST42:1998, 7.5.3), and after sterilization process failure (ANSI/AAMI ST42:1998, 7.6.6). See ANSI/AAMI ST42:1998, 7.6 for instructions on how to perform testing for installation and after major repair. See ANSI/AAMI ST42:1998, 7.7 for instructions on how to perform periodic monitoring of all types of packages and trays processed. See ANSI/AAMI ST42:1998, 7.6.6 for instructions on how to test the sterilization process after failures.

The user-assembled PCD should be routinely used at least once a week but preferably daily in each sterilizer (ANSI/AAMI ST42:1998, 7.5.3). If a sterilizer is designed for multiple types of cycles (e.g., wrapped items and flash-sterilized items), each sterilization mode should be tested (ANSI/AAMI ST42:1998, 7.6.1.2). Each load containing implantable devices should be monitored and, whenever possible, the implantable devices should be quarantined until the results of the biological indicator testing are available (ANSI/AAMI ST42:1998, 7.5.3). See ANSI/AAMI ST42:1998, 7.6 for instructions on how to perform the test.

6.4 PCDs for dry heat sterilization

6.4.1 Introduction

There are no universally accepted standardized PCDs for dry heat sterilizers. It is recommended that a representative package or tray that contains a biological indicator and that is conisdered the most difficult ot sterilze be selected from those most frequently processed and used as the PCD (ANSI/AAMI ST40:1992/(R)1998, 7.6.1). The use of a standard challenge test pack that included one chemical indicator and one biological indicator was proposed in ANSI/AAMI ST50:1995, 5.7.1.1., B.2, but this test pack was never validated and formally adopted.

6.4.2 Description

A PCD for a dry heat sterilization process should contain a biological indicator consisting of spores of *Bacillus subtilis* var. *niger* (now called *Bacillus atophaeus*) and a chemical indicator (ANSI/AAMI ST40:1992/(R)1998, 7.5.1 and ANSI/AAMI ST50:1995, 5.7.1.1). The test pack should provide a challenge to the sterilization process that is representative of the most difficult to sterilize item in the load being processed (ANSI/AAMI ST40:1992/(R)1998, 7.6.1).

6.4.3 Reference test packs

There are no reference test packs for dry heat sterilization processes. See ANSI/AAMI ST40:1992/(R)1998, 7.6.1 for further information. However, a proposed test pack consisting of nylon tubing, gauze sponges, biological indicator, and chemical indicator in a pouch is described in ANSI/AAMI ST50:1995, B.2.

6.4.4 Types of process challenge devices

6.4.4.1 Commercial process challenge devices

At the time of the publication of this TIR, the AAMI Process Challenge Device Working Group was not aware of any commercial PCDs on the market for dry heat sterilizers.

6.4.4.2 Health care facility-prepared process challenge devices

A health care facility-prepared PCD for dry heat sterilizers should create a greater challenge to the sterilization process than the load itself. The PCD should include a BI and CI. The PCD should be a package or tray that is representative of the same type of package or tray routinely processed through the sterilizer. The package or tray considered to be the most difficult to sterilize should be selected from those most frequently processed. Characteristics that should be considered when selecting challenge packs include multiple layers of dressing materials, large metal masses, and mixed packs incorporating both (ANSI/AAMI ST40:1992/(R)1998). The composition of the challenge test pack as described in ANSI/AAMI ST50:1995, B.2 should be considered.

6.4.5 Recommended practices—Application and frequency of use

The user-assembled PCD should be used for installation testing, after major repairs, and for periodic quality assurance testing (ANSI/AAMI ST40:1992/(R)1998, 7.5.2); the user-assembled PCD should also be used after sterilization process failures (ANSI/AAMI ST40:1992/(R)1998, 7.6.5). See ANSI/AAMI ST40:1992/(R) 1998, 7.6 for instructions on how to perform installation testing. See ANSI/AAMI ST40:1992/(R) 1998, 7.7 for instructions on how to perform periodic quality assurance testing of representative samples of actual product being sterilized. See ANSI/AAMI ST40:1992/(R)1998, 7.6.5 for procedures to follow after sterilization process failures.

The user-assembled PCD should also be used to monitor sterilization loads at least weekly (ANSI/AAMI ST40:1992/(R)1998, 7.5.2). Each load containing implantable devices should be monitored and, whenever possible, the implantable devices should be quarantined until the results of the biological indicator testing are available (ANSI/AAMI ST40:1992/(R)1998, 7.5.2). If the sterilizer is designed to be used for multiple types of cycle, then each sterilization cycle type should be tested (ANSI/AAMI ST40:1992/(R)1998, 7.6). See ANSI/AAMI ST40:1992/(R)1998, 7.6 for information on how to perform routine monitoring.

6.5 PCDs for hydrogen peroxide gas plasma sterilization

6.5.1 Introduction

At the time of the publication of this TIR, there were no published standards for PCDs used for challenge or routine monitoring of hydrogen peroxide gas plasma sterilization processes.

6.5.2 Description

A PCD for the hydrogen peroxide gas plasma sterilization process should contain a biological and chemical indicator. The test pack should provide a challenge to the sterilization process that is representative of the most difficult to sterilize item in the load being processed.

6.5.3 Reference test packs

There is no reference test pack for hydrogen peroxide gas plasma sterilization processes.

6.5.4 Types of process challenge devices

6.5.4.1 Commercial process challenge devices

A commercial, pre-assembled test pack is available for monitoring of the hydrogen peroxide gas plasma sterilization process.

6.5.4.2 Health care facility-prepared process challenge devices

A health care facility-prepared PCD for the hydrogen peroxide gas plasma sterilization process should create a greater challenge to the sterilization process than the load itself. The PCD should include a BI and CI and should be a package or tray routinely processed through the sterilizer that is considered the most difficult to sterilize item in the load being processed.

6.5.5 Recommended practices—Application and frequency of use

6.5.5.1 Challenge testing

A user-assembled PCD for the hydrogen peroxide gas plasma sterilization process or a commercially available test pack should be used as a challenge pack during initial installation testing and after major relocation, sterilizer malfunction, sterilization process failure, and any major repairs of the sterilizer. Three consecutive empty cycles using the test pack should be run in accordance with the sterilizer manufacturer's instructions.

6.5.5.2 Routine testing

AAMI recommends that this routine testing be done daily in a full load or each day that the sterilizer is used, and that the BI be placed in the load according to the BI manufacturer's instructions. The manufacturer of the commercially available test pack recommends that the hydrogen peroxide gas plasma sterilization process be tested frequently according to the health care facility's policy and procedures. The *Standards, recommended practices and guidelines* (AORN, 2003) recommends that low temperature hydrogen peroxide gas plasma spore testing should be performed at the same interval as other sterilizer testing in the facility.

Annex A
(informative)

Regulatory perspective

On May 28, 1976, the Food, Drug, and Cosmetic Act (FD&C Act) was amended to include medical devices. This new Medical Device Amendment granted the U.S. Food and Drug Administration (FDA) significant new regulatory authority to regulate medical devices to ensure their safety and effectiveness under their intended use. It required that all devices marketed in the United States for human use be classified into one of three regulatory classes ensuring that each device will be subject to controls that are appropriate for its class. Class I devices are the lowest risk devices and are subject to the general controls which apply to all medical device manufacturers such as establishment registration, device listing, compliance with the Quality System Regulation 21 CFR 820, premarket notification (510(k)) unless exempted, labeling, and medical device reporting. Class II devices pose relatively greater risk to patients and general controls are not sufficient. They require "special controls" to provide reasonable assurance of safety and effectiveness. Special controls, as defined by the FD&C Act, include performance standards, postmarket surveillance, patient registries, and development and dissemination of guidance documents. Class III devices are life-sustaining or life-supporting devices and require FDA premarket approval for ensuring their safety and effectiveness.

Process challenge devices are regulated by the FDA as an accessory to a medical device. Those PCDs that contain or are intended to be used with biological indicators (BIs) would be considered as an accessory to a BI, and those that contain or are to be used with chemical indicators (CIs) would be considered an accessory to a CI. Accordingly, they would be placed in the same regulatory class as the medical device to which they are considered an accessory.

The FDA has classified BIs and CIs as Class II medical devices requiring special controls to provide reasonable assurance of safety and effectiveness. Therefore, process challenge devices are also considered Class II devices. FDA defines a BI as "a device intended for use by a health care provider to accompany products being sterilized through a sterilization procedure and to monitor adequacy of sterilization. The device consists of a known number of microorganisms, of known resistance to the mode of sterilization, in or on a carrier and enclosed in a protective package. Subsequent growth or failure of the microorganisms to grow under suitable conditions indicates the adequacy of sterilization." A CI is defined as "a device intended for use by a health care provider to accompany products being sterilized through a sterilization procedure and to monitor one or more parameters of the sterilization process. The adequacy of the sterilization conditions as measured by these parameters is indicated by a visible change in the device."

Class II devices require the submission and receipt of clearance of a 510(k) premarket notification with the exception of those devices that have been exempted by federal regulation. In accordance with section 510(k) of the FD&C Act, during the 510(k) submission review, the FDA will make a determination of substantial equivalence of the new device as compared to a preamendment (pre-1976) device or devices that have received 510(k) clearance; that is, the FDA will determine whether the new device is as safe and effective as the predicate device. A substantial equivalence determination is not an FDA approval; rather, it is a marketing clearance. In the case of PCDs, the determination of substantial equivalence is based on the performance of the BI or CI within the PCD.

The FDA has cleared pre-assembled commercial PCDs for steam sterilizers and ethylene oxide sterilizers intended for use as routine test packs in health care settings. Some of these PCDs are marketed with or without the BI or CI for which they are intended. Test packs that are user-assembled do not require premarket clearance. Only PCDs that are intended for introduction into interstate commerce or commercial distribution require FDA clearance.

The gold standard for steam sterilization process challenge devices is the AAMI 16-towel test pack. The FDA accepts data demonstrating substantial equivalence to the 16-towel test pack or substantial equivalence to a legally marketed steam process challenge device. Similarly, data demonstrating substantial equivalence to the AAMI EO challenge test pack, AAMI EO routine test pack, or legally marketed EO test packs would be adequate for clearance.

Annex B
(informative)

The Bowie-Dick test pack

B.1 Introduction

The Bowie-Dick test was developed to detect air leaks and evaluate the ability of prevacuum sterilizers to reduce air residuals from the chamber space to a level sufficient to prevent air compaction by reentrainment into a load (the "small-load effect") as steam is introduced after evacuation. If there is insufficient air removal, either because the vacuum pump has malfunctioned, the control switch has cut off evacuation too soon, or chamber air leaks exist, steam will subsequently drive the available air back into the load, air pockets will occur, and sterilizing conditions will not be attained.

In 1963, publications by J.H. Bowie and his coworkers described a simple test suitable to determine if the vacuum system of a prevacuum sterilizer was functioning correctly (Bowie et.al., 1963a; Bowie et al., 1963b). A challenge device emerged to conduct the Bowie-Dick test.

The rationale for excluding the Bowie-Dick test pack from the scope of this TIR is that (a) the Bowie-Dick test pack does not meet the definition of a PCD as provided in the definition section of the TIR; and (b) the Bowie-Dick test pack evaluates the prevacuum phase of a steam sterilization cycle but does not monitor a sterilization process. Furthermore, ANSI/AAMI ST66 already addresses the Bowie-Dick test pack.

B.2 Description

Initially, measurements of vacuum system adequacy were made by placing thermocouples within a specified test pack, and in the chamber drain. In the publications by Bowie, the test sheet consisted of a piece of paper onto which a St. Andrew's cross was made with autoclave indicator tape. The test pack Bowie described was composed of Huckaback towels having a minimum size of 36 $\times$ 24 inches before laundering. These towels were folded into four along their length and then doubled across to give eight thicknesses of cloth. The number of towels will vary depending upon their thickness, but the stack should measure 10 to 11 inches high. When tested in a correctly functioning sterilizer with a holding time of 3.5 minutes at 134 °C with only the test pack in the chamber, the lines on the autoclave tape will change to a uniform dark color. The test measures steam penetration. Test failures may be due to inefficient air removal, air leakage into the chamber, or non-condensable gases in the steam supply. If operated properly, the sterilizer is capable of producing sterile goods. It is intended that this test be performed each day after the sterilizer is heated to operating temperature. Pre-printed test sheets were created because a recognized problem was the fact that the autoclave tape did not cover the entire test area and if the air pocket collected in a spot where there was no indicator tape, it would not be detected. In pre-fabricated test packs, other chemical indicators are also used as the detection method for the presence of air during the test.

B.3 Reference test packs

The test pack in ANSI/AAMI ST46:2002, *Steam sterilization and sterility assurance in health care facilities*, is described as follows:

> The Bowie-Dick test pack consists of folded huck or absorbent surgical towels. The towels should be freshly laundered, but not ironed. The towels must be folded to a size not smaller than 9 inches (23 centimeters) in one direction and 12 inches (30 centimeter) in the other direction, and placed one above another. The height of the test pack must be 10 to 11 inches (25 to 28 centimeters). The total number of towels may vary from test to test, depending on towel thickness and wear. A commercially available Bowie-Dick-type test sheet or inhospital-constructed, crossed indicator tape (configured in the shape of an X) must be placed in the center of the pack. Caution should be exercised in selecting test materials that may bias the test favorably with respect to the air reentrainment principle by preventing the reaccess of air from all directions. If test sheets are used, for example, it should be determined from the manufacturer whether their porosity equals or exceeds that of the stacked towels. The sensitivity of the indicating ink should also be ascertained. Some test materials may not reveal marginally poor conditions. A single wrap, loosely applied, is used (woven or non-woven).

Test packs or devices other than those described above, such as disposable Bowie-Dick-type test packs, may be used only if in scientific experiments they have proven to be equivalent.

B.4 Types of test packs for the Bowie-Dick test

B.4.1 Commercial Bowie-Dick test packs

Although health care facilities were provided detailed information from AAMI on how to prepare Bowie-Dick test packs, the preparation of these packs was time-consuming and pack performance varied depending on how the packs were prepared and the source of materials that made up the packs. This led to the development and availability of several commercial, prefabricated Bowie-Dick-type test packs. These test packs are designed to create a challenge that is equivalent to the AAMI test pack. Check with the manufacturer of the commercially available test pack for performance data and instructions for use.

B.4.2 Health care facility-prepared Bowie-Dick test packs

The AAMI Bowie-Dick test pack that can be prepared entirely by the health care facility is described in B.3.

B.5 Recommended practices—Application and frequency of use

The Bowie-Dick test must be carried out each day the sterilizer is used, before the first processed load. A shortened cycle (i.e., a cycle omitting the postvacuum drying phase) should be run first to properly heat the sterilizer. If the sterilizer is used continuously, the test may be made at any time, but should be made at the same time every day.

The conventional test pack must be placed horizontally in the front, bottom section of the sterilizer rack, near the door and over the drain, in an otherwise empty chamber. The commercial test pack should also be placed over the drain in an otherwise empty chamber, but the orientation recommended by the manufacturer should be followed.

A prevacuum cycle is run as specified by the sterilizer manufacturer. The recommended holding time is 3.5 minutes, but if half-minute exposures cannot be selected on the sterilizer, a 4 minute holding time may be used. The holding time must never exceed 4 minutes at 134 °C (273 °F). Drying may be omitted to save time without affecting the outcome of the test. When removed from the sterilizer, the test pack may still be hot and should be opened carefully to avoid thermal injury to the hands or face. The test sheet or crossed indicator tape must be removed from the pack and examined by a person trained in its interpretation.

After a satisfactory test run in a towel test pack, the tape or printed test sheet should show a uniform color change; i.e., the color in the center should be the same as that at the edges. The exact color change of the tape or sheet may depend on brand or storage conditions; of importance is whether the same color occurs uniformly from the center through the edges. For a commercially available test pack, follow the instructions of the manufacturer with respect to a satisfactory interpretation.

Any unexpected color change, such as the center of the sheet or crossed indicator tape being paler than the edges, indicates that there was an air pocket present during the cycle due to sterilizer malfunction. Also, as was the case for a commercially available test pack with a satisfactory test, follow the instructions of the manufacturer with respect to a result that is not satisfactory. Any indication of a malfunction must be reported to the supervisor on duty, who will determine the disposition of the sterilizer (i.e., whether it should be retested, serviced, or remain in use).

B.6 Standards

B.6.1 International Standards

Three standards have been developed for the Bowie-Dick test. The numbers and titles are:

— ISO 11140-3:2000, *Sterilization of health care products—Chemical indicators—Part 3: Class 2 indicators for steam penetration test sheets*

— ISO 11140-4:2001, *Sterilization of health care products—Chemical indicators—Part 4: Class 2 indicators for steam penetration test packs*

— ISO 11140-5:2000, *Sterilization of health care products—Chemical indicators—Part 5: Class 2 indicators for air removal test sheets and packs*

These documents have been developed due to different parts of the world interpreting the original work by Dr. Bowie in different ways. Some countries refer to the test as an air removal test and follow specific criteria for a pass response, while others consider it a steam penetration test with specific acceptance criteria. In reality, both are needed. A sterilizer cannot achieve acceptable steam penetration without air removal and air removal cannot be achieved without adequate steam penetration. In all instances, the disposable/alternative test pack is compared to the cotton sheet or cotton towel pack. The following list attempts to document the differences in materials and interpretation between these standards.

Table B.1—Comparison of ISO 11140 standards

	ISO 11140-3	ISO 11140-4	ISO 11140-5
Test pack material	Cotton sheets (185 ± 5) g/m^2 warp (30 ± 6) weft (27 ± 5)	Cotton sheets (185 ± 5) g/m^2 warp (30 ± 6) weft (27 ± 5)	100 % cotton surgical towels
Test pack density	0.42 kg/dm^3	0.42 kg/dm^3	0.20 kg/dm^3
Test pack size	22 × 30 × 25 cm	22 × 30 × 25 cm	9 × 12 × 10 to 11 inches
Acceptable criteria (See the appropriate standards for complete descriptions of "pass" and "fail" conditions.)	Must show pass: Temp in pack not less than 0.5 °C lower than drain temp throughout plateau. Must show fail: Temp in pack between 2 °C and 3 °C lower than drain at start of plateau, exclusive of a 15 second equilibration time.	Must show pass: Temp in pack not more than 1 °C lower (for first 30 seconds) than drain temp; no difference for remainder of plateau. Must show fail: Temp in pack must be more than 2 °C lower, but less than 5 °C lower, than drain temp at start of plateau. Temp in pack at end of plateau shall not be more than 1°C cooler than drain temp.	Must show pass: When temp measured in drain has attained 134 °C, difference between measured temp in drain and center of test pack shall be less than 0.5 °C and shall remain so for duration of 3.5 minute exposure time, exclusive of a 15 second equilibration time. Must show fail: 2 °C difference between drain and center of pack 1 minute before end of 3.5 minute, 134 °C cycle.

ISO 11140-3 gives a method for evaluating test sheets, which are used within the standard 7 kg towel pack specified in the standard. Only the indicator requires testing; the performance of the towel pack has previously been demonstrated (Bowie et al., 1963). The method chosen to induce a failure is one of air injection; although this method is artificial with respect to a genuine sterilizer fault, it does allow reproducible creation of a 2 °C to 3 °C temperature depression in the center of the towel pack.

ISO 11140-4 gives a range of methods for testing alternative test packs, which may be single use or reusable. Unlike ISO 11140-3, where only the indicator within the pack (challenge device) is to be tested, ISO 11140-4 requires that the performance of the indicator in combination with its test load have equivalent performance to the reference 7 kg towel pack. As the challenge device in combination with the indicator is now being tested, a range of induced failure methods are used to characterize the performance of the system as a whole, and to ensure equivalence to the reference towel pack. Some alternative products may appear to be equivalent to the reference towel pack by one particular induced failure method, but may not detect failures by another method due to inadequacies of the challenge device, indicator, or both.

The three methods required in ISO 11140-4 are air injection, induced air leak, and a modified air removal stage. Air injection is similar to the method stated in ISO 11140-3, and involves injecting a specified quantity of air into the sterilizer chamber using a pneumatic cylinder. Simply allowing a quantified amount of atmospheric air to leak into the sterilizer while the chamber is below atmospheric pressure creates an induced leak. This is designed to simulate a door gasket failure, for example. Modification of the air removal stage is achieved by altering the number and levels of the steam/vacuum pulses used to effect steam penetration into a porous load. This will simulate a faulty sterilizer pressure switch or incorrectly functioning pressure transducer.

ISO 11140-5 provides a method for evaluating indicator test sheets and pre-assembled test packs. It requires that a fault condition (fail result) be created by inadequate air removal. An inadequate vacuum condition is found where the 100 % cotton surgical towel test pack will exhibit a 2 °C temperature difference between the drain and the center of the pack 1 minute before the end of a 3.5 minute, 134 °C cycle. A test sheet within the test pack must show an air pocket when the test pack is subjected to the same inadequate vacuum condition. Similarly, a pre-assembled test pack must show an air pocket when tested with the same fault condition. Neither the indicator test sheet nor the test pack is required to be tested by air leakage or air injection.

B.6.2 AAMI standards

ANSI/AAMI ST66 is substantially the same as the ISO 11140-5 standard that is described in B.2.1.

B.7 Current trends

There are also challenge devices for Bowie-Dick testing that do not use an indicator sheet, but instead utilize temperature sensors. The pass or fail result is determined by the interaction of the temperature measurements and the software of the device.

To augment Bowie-Dick testing, some sterilizers are now fitted with software systems that can determine if an air leak is present within the sterilizer. In addition, mechanical tests can be run to determine if non-condensable gases are leaking into the sterilizer.

It is clear that the Bowie-Dick test has evolved from its inception when Dr. Bowie and others first developed the test in the early 1960s. The science of sterilization will continue to evolve in the years to come and monitoring will likely evolve simultaneously.

Annex C
(informative)

International perspectives on process challenge devices

C.1 Introduction

European standards concerning sterilization process challenges for validation of sterilizer performance differ from those generally accepted in the United States and Canada. Two types of test packs are defined: one is similar in construction to the U.S. standard reference packs (fabric materials), and the other uses long tubes (helices) as a diffusion challenge. Interestingly, the physics behind the two approaches is similar, but the execution of that physics is markedly different.

C.2 Basic principles

Any sterilization process requires that the sterilizing agent penetrate the load to be sterilized to deliver adequate sterilizing conditions to all portions of that load. This is true for all sterilization processes and can be modeled using a test load or PCD. PCDs are defined as being representative of a typical load, or providing a challenge to the process that is significantly greater than a typical load, much as BIs are more challenging to a process than typical bioburden.

PCDs present a penetration challenge to the sterilization process for which they are designed. There are different issues in achieving penetration for each process, and thus PCDs are process-specific. However, all PCDs present a challenge to diffusion of the sterilizing agent to the test object (i.e., a biological or chemical indicator). Instrumentation could perform the function of a PCD by measuring the appropriate process parameters in the challenge volume, but this is not yet a standard practice.

Since PCDs are to present a diffusion challenge to a process, the construction of the PCD must create a repeatable challenge that will not vary between uses if it is a reusable design, or within production variation from PCD to PCD of the same model or disposable design.

C.3 United States/Canadian PCDs

In the United States and Canada, PCDs are designed to mimic a challenging hospital load. This attribute is discussed elsewhere in this document and will not be discussed at any length here. However, it must be noted that the standard PCDs for steam sterilization to which commercial product is an equivalent use multiple fabric towels as much of the diffusion challenge. The porosity of these items will change as they age and are reused, influencing the diffusion of sterilizing agent within the PCD. The standard PCDs for EO sterilization, to which commercial product is equivalent, use towels for moisture absorption and heat sink, and use diffusion resistors (plastic syringes or glass syringes without the protective cap) as much of the diffusion challenge.

In 1977, the Canadian Standards Association published *Effective Sterilization in Hospitals by the Ethylene Oxide Process* (CSA Standard Z314.2-M1977). The installation challenge test pack and routine challenge pack for EO sterilization processes defined in this document are identical to the AAMI challenge and routine test packs.

AAMI began working on developing a recommended practice in the late 1970s. In 1980, the draft document *Good hospital practice: Ethylene oxide sterilizers—EO test pack challenge* (draft; AAMI EO-TPC-D 7/80) was developed. The consensus of the AAMI EO Sterilization Hospital Practices Working Group was that additional data needed to be developed before they could finalize a recommendation, as other published information was lacking sufficient data.

In 1982, the first edition of *Ethylene Oxide Use in Hospitals: A Manual for Health Care Personnel* was published by the American Society for Hospital Central Service Personnel of the American Hospital Association (ASHCSP). The recommendations outlined in this document were adapted from the AAMI 1980 draft document, the CSA 1977 document, and others. At that time, both organizations were in the process of making revisions and conducting additional research. The components of the test packs were selected to challenge the parameters of the EO process: towels and absorbent cotton for moisture absorption and heat sink; rubber and/or plastic for EO absorption; glass test tubes with plastic (polypropylene) cap, loosely applied, as heat sinks; diffusion resistors (plastic or glass syringes without protective caps may be used); and biological and chemical indicators.

In 1985, subsequent to extensive round-robin laboratory testing of the routine hospital monitoring test pack, the AAMI working group finalized their recommendations in *Good hospital practice: Performance evaluation of ethylene oxide sterilizers—Ethylene oxide test packs* (AAMI EOTP 2/85). Based on laboratory analysis with the routine monitoring test pack, the consensus was to standardize the challenge test pack by incorporating cotton surgical

towels. The number of towels recommended was four towels, which would present a sufficient challenge to the sterilizer for EO penetration. Round-robin laboratory test results were published in the *AORN Journal* in June 1993 (Hart, et al., 1993). The 1985 document on ethylene oxide test packs was subsequently incorporated in the AAMI recommended practice (*Good hospital practice—Ethylene oxide sterilization and sterility assurance*, ANSI/AAMI ST41:1992). The recommendation has not changed over the years and is published in the latest edition.

In 1986, the ASHCSP's *Ethylene Oxide Use In Hospitals: A Manual for Health Care Personnel* revised the recommendations published in 1982 based on the research and recommendations of AAMI. No major issue was noted in the use of the recommended challenge test packs of 1982. The major issue then, as it is today, is the environmental humidity level maintained in a health care facility processing department. If the environmental humidity level is low, positive BIs will result. The recommendations in the third edition of this manual (Danielson, 1998) follow the same basic principles as were recommended in the previous editions.

C.4 Australian PCDs

In 1975, the Standards Association of Australia published *Ethylene oxide sterilizers (using ethylene oxide/dichlorodifluoromethane 12 %/88 % m/m sterilizing gas mixture)*. Under 5.5, Sterilizing test, and 5.5.1, Method of test, spore strips prepared with a suitable strain of *Bacillus subtilis* var. *niger* shall be placed in the most inaccessible portion of the standard test pack and the normal sterilizing cycle shall be carried out (see Appendix C8, Standard challenge pack). Agreement has not yet been reached on a suitable standard challenge pack. Until one has been developed, the purchaser should nominate the pack to be used in the test, taking into consideration the items to be sterilized.

C.5 European PCDs

EN standards take a variety of approaches to creating a diffusion challenge for a given sterilization process to overcome. These approaches include fabric-based test packs (EN866-3, EN867-3, and EN867-5) and tubular diffusion challenges, or helices, along which the sterilizing agent must migrate to reach the indicator. The fabric-based test packs differ from the U.S. fabric-based test packs in the details of their construction, but not in principle of action. Therefore, they are not discussed here. The helix approach was first published by Line and Pickerell (1973) for low-temperature steam-formaldehyde cycles, which are not used in the U.S. Helical devices have the advantage that they provide a repeatable challenge to a process, and that repeated use of the PCD would not change its resistance to diffusion, unless the tubing is bent, straightened, or otherwise changed in configuration. The disadvantage, from a U.S. viewpoint, is that these devices bear essentially no resemblance to the loads being processed by a sterilizer.

C.5.1 EN1422—Ethylene oxide

The PCD for ethylene oxide sterilizer validation is defined in EN1422. This is a tubular construction of stainless steel tubing with an inner diameter of 3 mm (0.118 inches) and a length of 4.55 m (14 feet 11.13 inches or simply 15 feet). The actual figure of merit is a length-to-bore ratio of ~1500:1. This is coiled into a helix that has one end open and the other attached to a capsule that contains the test object. The test system (tubing and capsule) must have a volume of 32 mL, of which that capsule has a volume of 0.85 mL.

This definition is very precise, except that it does not determine the radius of the helix, and thus the diffusion resistance is not defined precisely (or at all) between the limits of flow through a straight tube and a tightly coiled design of minimum radius. The flow or diffusion through a tube, and thus its diffusion resistance, depends strongly on the radius of any bends in the coil, with the straight tube providing the low-resistance limit and the coiled tube providing the high-resistance limit.

C.5.2 EN867-5—Steam

The helix PCD that is used to validate a steam sterilization process is defined in this document for small sterilizers. It is similar to the EN1422 PCD for ethylene oxide in concept, but differs in construction. This helix is made of PTFE (polytetrafluoroethylene) tubing, of 2 mm ID, 0.5 mm wall thickness, with a length of 1500 mm. The mass of the capsule containing the indicator is 10 g, and the capsule volume is 6 % of the volume of the tubing portion of the helix. This design also does not specify the radius of the helix, and thus leaves the actual diffusion resistance not fully specified.

C.6 ISO standard PCDs

The International Standards Organization (ISO) standards generally recognize all approaches, deferring to national standards for details of construction.

C.7 Summary

ISO and EN standards provide alternative PCD designs to those that are typically used in the United States and Canada. These designs fall into two categories: cotton-based packs and helices. The cotton-based packs are philosophically identical to the United States/Canada standards, but differ in construction. The helices are markedly different from those in the United States and Canadian standards, although, with proper specification, they provide a more consistent challenge to a sterilization process than fabric-based systems provide. The specification for preparing these devices is not complete in the current ISO and EN standards. Consequently, results generated using these devices may be subject to some dispute. Further, their marked difference from real hospital loads in construction and appearance makes their acceptance in the United States/Canada questionable.

Annex D
(informative)

Development and qualification of the 16-towel test pack used for steam sterilization

D.1 Introduction

Through a cooperative effort among hospital personnel, industrial representatives, and independent consultants, testing was conducted to develop a new biological indicator test pack for evaluation of steam sterilizers within health care facilities. The new test pack was to have performance characteristics similar to the old test pack and consist of materials readily available to hospital personnel. This section summarizes the testing that resulted in the new 16-towel test pack being recommended as an alternative to the original test pack in the second edition of the recommended practice (AAMI, 1988), and recommended here as the sole challenge pack. The 16-towel test pack became the 'gold standard' or reference BI test pack for challenging steam sterilization conditions.

D.2 Survey and preliminary testing

Before any laboratory testing, a questionnaire was distributed to health care personnel to solicit their thoughts on the original 12 × 12 × 20 inch pack and their ideas concerning a new test pack. Results of the questionnaire confirmed that most hospitals did not have available all of the materials to make the 12 × 12 × 20 inch pack, since they were purchasing such items as lap sponges as sterile, single-use items. The majority of the respondents indicated that they wanted a test pack that was well defined in terms of content, size, and biological indicator placement. Surgical towels were identified as the material most readily available within health care facilities for making a test pack. Because surgical towels were also used in the Bowie-Dick test pack and recommended for use in EO test packs (AAMI, 1985), it was decided to investigate the use of surgical towels for the biological indicator test pack for the steam sterilization process.

Questions arose about the variability of surgical towels used by health care facilities and how this might affect test pack performance. More than 20 test packs were obtained from health care facilities throughout the country. All towels had been washed and were in routine use at the various institutions. Average surgical towel dimensions were 16.5 by 26.3 inches.

In considering the characteristics of the 12 × 12 × 20 inch heterogeneous pack, it was noted that the materials were arranged in two stacks with a space between. The two stacks act as virtually independent challenges to air evacuation and steam penetration, as measured by temperature profiles, although they are contained in the same wrapper. Preliminary testing was conducted in a 250 °F (121 °C) gravity cycle to determine the number of towels and size of test pack needed to yield performance characteristics similar to those of the 12 × 12 × 20 inch pack.

Figure D.1 shows temperature profiles from 12 × 12 × 20 inch packs prepared and run at two different test laboratories. Significantly different profiles were observed, although both laboratories prepared their packs in accordance with the 1980 AAMI recommendations. The packs differed in size of wrapper used, method of folding towels, and type of surgical gowns used. None of these parameters was specified in descriptions of the 12 × 12 × 20 inch pack.

It was agreed that the performance of the new towel pack should approximate that of the slower-to-heat 12 × 12 × 20 inch pack illustrated in Figure D.1. The preliminary testing indicated that 16 surgical towels folded to produce a pack with overall pack dimensions of 9 × 9 × 6 inches yielded thermal come-up profiles and biological indicator results comparable to the 12 × 12 × 20 inch pack with the slowest heat-up time.

Tests were run to compare horizontal (flat) versus vertical (on edge) placement of the towel pack. As expected, horizontal placement provided more of a challenge to sterilization in a gravity cycle, as indicated by a longer come-up time (1 minute to 2 minutes) and the biological indicator test results. Tests were also run with the towel pack in a fully loaded chamber and with the towel pack in an otherwise empty chamber. The use of a single pack was more of a challenge to the sterilizer because the chamber come-up time was quicker, thereby activating the exposure timer for initiation of the sterilization phase of the cycle sooner. The center of the pack, on the other hand, took the same time to reach temperature whether the chamber contained one pack or was fully loaded.

Table D.1 summarizes characteristics of the 16-towel packs that were tested. The average pack dimensions were 9.4 × 8.9 × 6.1 inches. The average weight and density of the packs were 3.3 pounds and 11.3 pounds per cubic foot, respectively. Questions arose concerning the differences between huck and absorbent surgical towels used to make up a 16-towel pack. Figure D.2 shows the average temperature profiles in a gravity cycle for the two types of packs. No significant differences were observed.

Table D.1—16-towel pack survey

Towel size		Average pack size			Average pack weight (lbs.)	Average pack density (lbs/ft^3)
Length (in)	Width (in)	Length (in)	Width (in)	Height (in)		
26.3 ± 2.1*	16.5 ± 1.3*	9.4	8.9	6.1	3.3**	11.3

* Mean ± 1 S.D.
** Pack weights ranged from 2.6 lbs. to 3.7 lbs.

D.3 Validation testing

D.3.1 Validation testing in gravity cycles

The 16-towel test packs were processed in 250 °F (121 °C) gravity cycles. Thermocouples and biological indicators were placed in the center of each pack. The 12 × 12 × 20 inch packs were similarly instrumented to permit a direct comparison of the two types of packs. The 12 × 12 × 20 inch packs were placed vertically (on edge) in the sterilizer, and the 16-towel packs were placed horizontally (flat). The packs were evaluated at three different laboratories. Figure D.3 shows the average temperature profile for the 16-towel pack, which is very similar to the profile shown in Figure D.1 for the slowest-to-heat 12 × 12 × 20 inch pack. The pack-to-pack variation for the 16-towel pack was significantly less than for the 12 × 12 × 20 inch pack, as evidenced by the standard deviations. Table D.2 shows the biological-indicator results; the 16-towel pack was more resistant than the 12 × 12 × 20 inch pack in a 250 °F (121 °C) gravity cycle.

Table D.2—Biological-indicator results from 250 °F (121 °C) gravity cycle

Exposure time (minutes)	Biological-indicator response*			
	12 × 12 × 20 inch pack		16-towel pack	
	Spore strips			
16	NT**		4/4	(100 %)
18	2/2	(100 %)	1/4	(25 %)
20	5/12	(42 %)	8/16	(50 %)
22		(75 %)	NT**	
25	0/12	(0 %)	0/10	(0 %)
	Self-contained			
16	NT**		5/8	(63 %)
18	4/4	(100 %)	2/8	(25 %)
20	11/16	(69 %)	7/24	(29 %)
22	4/8	(50 %)	NT**	
25	0/16	(0 %)	0/12	(0 %)

* Number positive/number exposed (% positive)
** Not tested

D.3.2 Validation testing in vacuum-assisted cycles

Both deep-vacuum and pulsing vacuum-assisted sterilizers were used for the evaluations. In general, the center-of-pack temperatures closely followed the sterilizer drain line temperature. The temperature profiles of the 12 × 12 × 20 inch pack and the 16-towel pack were identical, or the 16-towel pack lagged behind the 12 × 12 × 20 inch pack by a maximum of 30 seconds. Table D.3 summarizes the biological indicator results from a deep-vacuum sterilizer. Spore strips were sterile with exposure times of two minutes or less, and self-contained indicators were killed with exposure times of four minutes. The data indicates that in vacuum-assisted cycles, the 16-towel pack is slightly more resistant than the 12 × 12 × 20 inch pack. Table D.4 summarizes the biological-indicator results when test packs were run in a pulsing vacuum cycle at 270 °F (132 °C). As with the deep-vacuum cycle, the 16-towel pack was slightly more resistant.

Table D.3—Biological indicator results from 270 °F (132 °C) deep-vacuum cycle

Exposure time (minutes)	Biological-indicator response*			
	12 × 12 × 20 inch pack		16-towel pack	
	Spore strips			
0	3/4	(75 %)	4/4	(100 %)
0.5	5/14	(36 %)	4/18	(22 %)
2	0/18	(0 %)	0/18	(0 %)
3	0/16	(0 %)	0/16	(0 %)
4**	0/16	(0 %)	0/16	(0 %)
	Self-contained			
0	8/8	(100 %)	8/8	(100 %)
0.5	20/28	(71 %)	26/28	(93 %)
2	4/28	(14 %)	14/28	(50 %)
3	5/32	(16 %)	11/32	(34 %)
4**	0/32	(0 %)	0/32	(0 %)

* Number positive/number exposed (% positive)
** Recommended exposure

Table D.4—Biological-indicator results from 270 ºF (132 ºC) pulsing vacuum cycle

Exposure time (minutes)	Biological-indicator response*			
	12 × 12 × 20 inch pack		16-towel pack	
	Spore strips			
1	1/9	(11 %)	0/17	(0 %)
2	0/3	(0 %)	1/16	(6 %)
3	0/4	(0 %)	0/14	(0 %)
	Self-contained			
1	3/17	(18 %)	7/28	(25 %)
2	0/11	(0 %)	5/31	(16 %)
3	0/8	(0 %)	0/22	(0 %)

* Number positive/number exposed (% positive)

D.4 Direct comparison of the 12 × 12 × 20 inch and 16-towel test packs

The foregoing biological indicator and thermocouple testing was conducted with each test pack placed individually in an otherwise empty chamber. To reduce some of the cycle-to-cycle variation inherent in the testing, a final series of test cycles was run with both a 16-towel pack and a 12 × 12 × 20 inch pack present in the chamber at the same time.

In one test series, five biological indicators were used per test pack. After exposure to the sterilization cycle, three of the five biological indicators were cultured for sterility and two were assessed by the most probable number (MPN) technique, as described in *United States Pharmacopeia* (1984).

In the second test series, all five biological indicators were cultured for sterility after exposure, and three chemical indicators were scored on a ranking scale. The ranking scale was 0 to 13, with 13 equal to a complete change of the

chemical indicator. A thermocouple was located approximately two inches from the chamber drain, and temperature readings were taken at one minute intervals to calculate a F_o value for each cycle.

The results of the first and second series of tests are shown in Tables D.5 and D.6, respectively. The data shown in Table D.6 was evaluated statistically to determine if performance between the two packs differed significantly. An F test showed homogeneity of variance for both the fraction-value and chemical indicator data. A series of paired or unpaired t-tests using data with F_o values in the range of 18 minutes to 27 minutes or 26 minute ± 1 minute showed no significant differences between the 16-towel pack and the 12 × 12 × 20 inch pack (t = 0.124 to 0.402, p 0.05, 4 or 5 d.f.). The Mann-Whitney U-test also showed no significant differences between the two types of pack (p 0.35, $n_1 = n_2 = 5$, U = 10). There was minimal correlation between the independent variables (steam exposure time or F_o value) and the dependent variables (fraction-value or chemical-indicator results), with t-values in the range of 0.176 to 0.834 (p 0.5 to 0.1, 3 d.f.).

Overall, this data provides little or no support for a rejection of the null hypothesis of no difference between the 16-towel test pack and the 12 × 12 × 20 inch test pack at the p = 0.1 level; that is, no statistically significant differences were found in the performance of the two packs.

D.5 Summary of round-robin testing

The results of testing showed significant variation in the performance of the 12 × 12 × 20 inch pack, depending on how the pack was constructed. Overall, the 16-towel pack performed similarly to one of the more difficult configurations of the 12 × 12 × 20 inch pack. Although the two types of packs differed somewhat in specific types of sterilization cycles, the 16-towel pack showed less run-to-run variation. The committee decided to recommend the 16-towel pack for use in biological monitoring, because the 16-towel pack gives more reproducible results and can be more easily constructed than the 12 × 12 × 20 inch pack.

Table D.5—Comparison of the 16-towel pack with the 12 × 12 × 20 inch pack by most probable number and sterility assessment of spore strips in a 250 °F (121 °C) gravity cycle*

Exposure time (at 250 °F)	Most probable number assessment			Sterility assessment (survivors/# tested)
	Spore strip	Suspending fluid	MPN value	
14 minutes				
16-towel pack	#1	+	800	3/3
	#2	+	460	
12 × 12 × 20 inch pack**	#1	+	460	3/3
	#2	+	3,000	
15 minutes				
16-towel pack	#1	+	460	1/3
	#2	+	< 460	
12 × 12 × 20 inch pack**	#1	+	460	2/3
	#2	+	460	
16 minutes				
16-towel pack	#1	+	460	NT
	#2	+	< 460	
12 × 12 × 20 inch pack**	#1	+	460	NT
	#2	+	460	

* Noncollaborative data gathered by an independent laboratory
** 52 × 52 inch wrap

Table D.6—Fraction-negative results in a 250 °F (121 °C) gravity cycle*

F_o value	Intended exposure time at 250 °F (minutes)	16-towel pack				12 × 12 × 20 inch pack**			
		Spore strip	Chemical indicator***			Spore strip	Chemical indicator***		
			1	2	3		1	2	3
18.8	16	4/5	0	0	0	5/5	1	9	2
25.7	15	3/5	4.5	5	4	1/5	11	9.5	8
26.2	18	4/5	12	9	8.5	5/5	7	6	12
26.4	17	5/5	4	4	11	3/5	2	2	3
26.8	19	2/5	13	12	9	2/5	7	4	6
Total		18/25	96.5			16/25	89.5		

* Noncollaborative data gathered by an independent laboratory
** 50 × 64 inch wrap
*** Scale of chemical indicator response: 0 = no evidence of sterilization; 13 = complete response, indicating sterilization conditions met

D.6 Supplemental data for steam-flush pressure-pulse cycles

After the round-robin testing to qualify the 16-towel test pack, noncollaborative data was collected to compare the 16-towel test pack and the 12 × 12 × 20 inch test pack in steam-flush pressure-pulse cycles. The two types of test packs were processed in 250 °F (121 °C) cycles. Biological indicators were placed in the center of each pack. The two packs were placed horizontally (flat) in the sterilizer. There was no discernible difference between the two packs in biological indicator results, since all of the biological indicators were killed in similar exposure times (Table D.7).

Similar testing was performed for 270 °F (132 °C) steam-flush pressure-pulse cycles. Spore strips were found to be sterile after exposure times of 0.5 minutes or more. Self-contained biological indicators were killed with exposure times of two minutes or more. There was no discernible difference between the two packs in microbial kill (Table D.8).

Table D.7—Biological-indicator results from 250 °F (121 °C) steam-flush pressure-pulse cycle*

Exposure time (minutes)	Biological-indicator response**			
	12 × 12 × 20 inch pack		16-towel pack	
	Spore strips			
8	17/18	(94.4 %)	18/18	(100 %)
10	0/18	(0 %)	0/18	(0 %)
12	0/18	(0 %)	0/18	(0 %)
14	0/18	(0 %)	0/18	(0 %)
	Self-contained			
8	18/18	(100 %)	18/18	(100 %)
10	3/18	(16.6 %)	6/18	(33.3 %)
12	0/18	(0 %)	0/18	(0 %)
14	0/18	(0 %)	0/18	(0 %)

* Noncollaborative data gathered by an independent laboratory
** Number positive/number exposed (% positive)

Table D.8—Biological-indicator results from 270 °F (132 °C) steam-flush pressure-pulse cycle*

Exposure time (minutes)	Biological-indicator response**			
	12 × 12 × 20 inch pack		16-towel pack	
	Spore strips			
0.5	0/18	(0 %)	0/18	(0 %)
2	0/18	(0 %)	0/18	(0 %)
3	0/18	(0 %)	0/18	(0 %)
4	0/18	(0 %)	0/18	(0 %)
	Self-contained			
0.5	18/18	(100 %)	18/18	(100 %)
2	0/18	(0 %)	0/18	(0 %)
3	0/18	(0 %)	0/18	(0 %)
4	0/18	(0 %)	0/18	(0 %)

* Noncollaborative data gathered by an independent laboratory
** Number positive/number exposed (% positive)

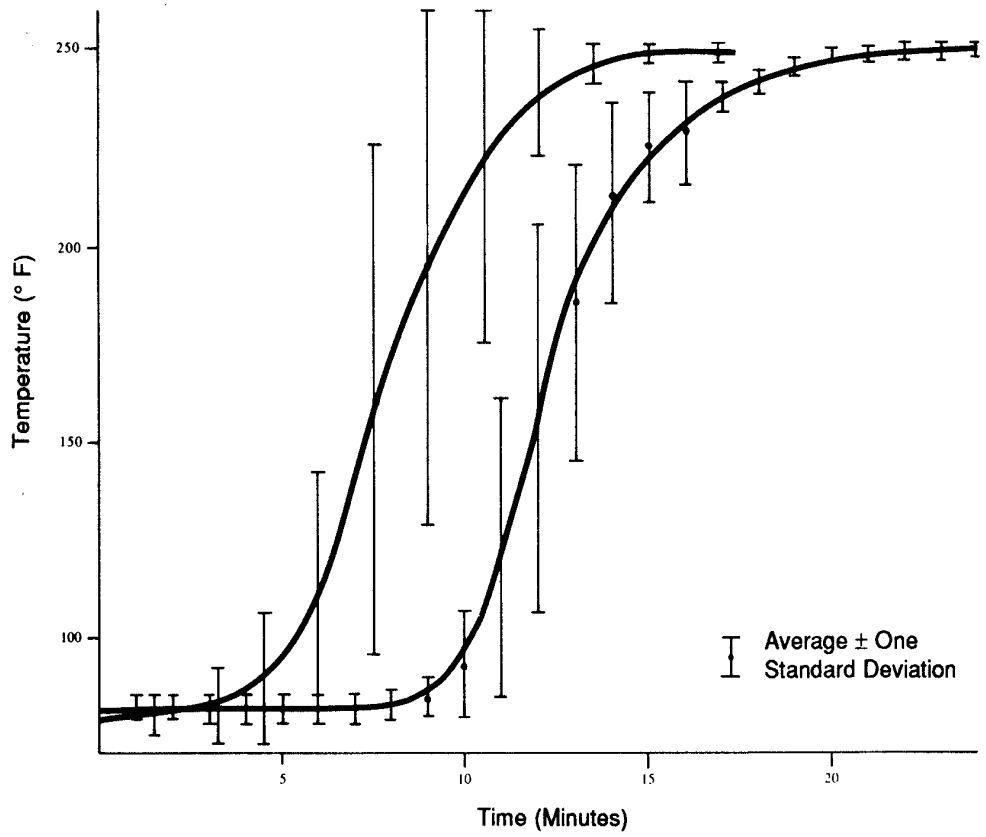

Figure D.1—Temperature profiles performed at two different laboratories of 12 × 12 × 20 inch test packs in a 250 °F (121 °C) gravity cycle

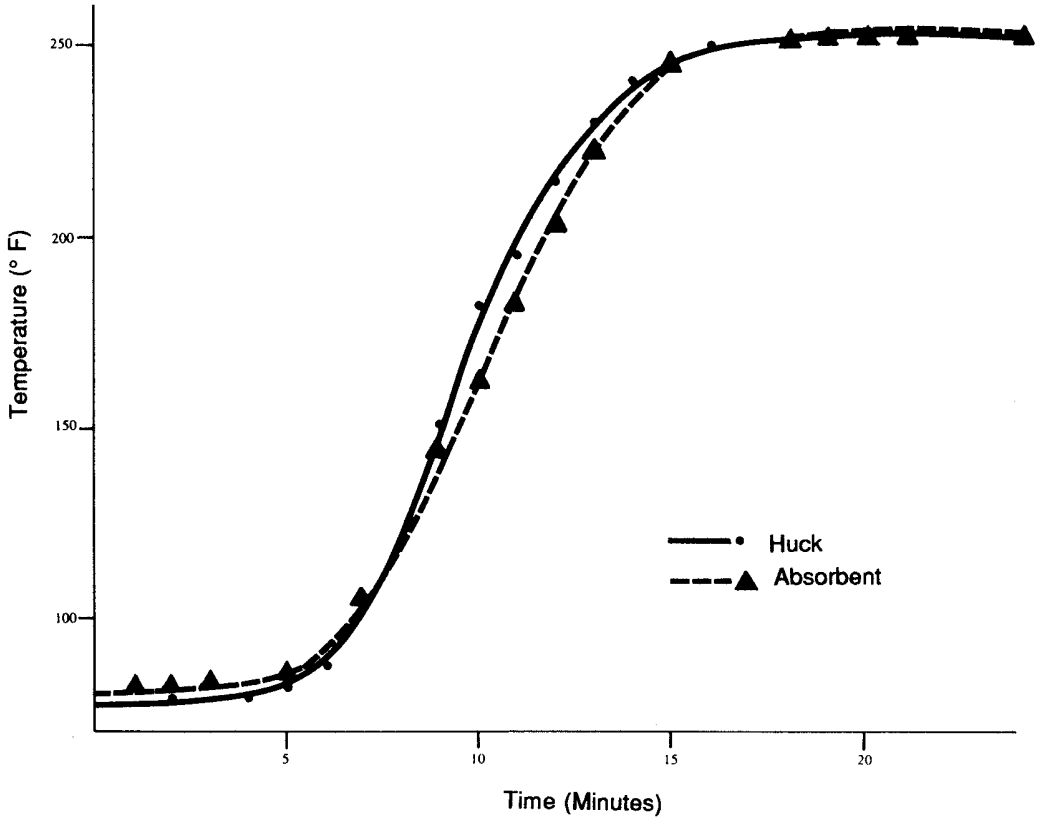

Figure D.2—Temperature profiles for huck and absorbent 16-towel packs in a 250 °F (121 °C) gravity cycle

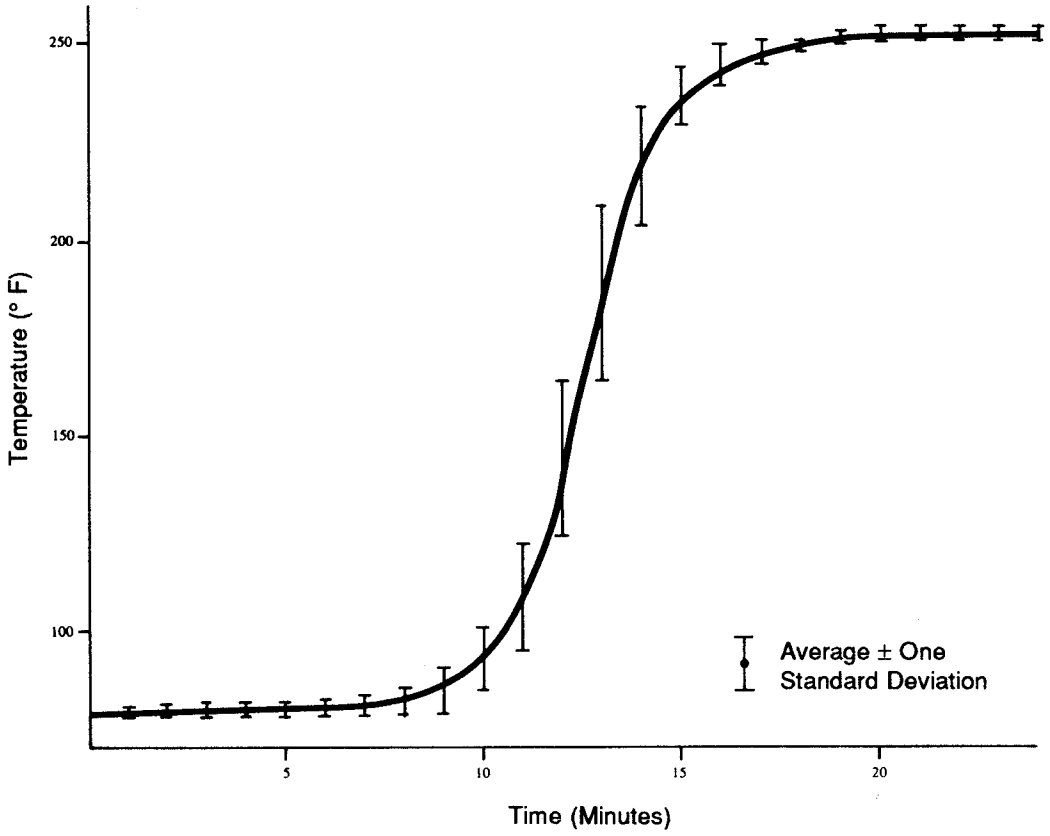

Figure D.3—Average temperature profile for the 16-towel pack in a 250 °F (121 °C) gravity cycle performed at three different laboratories

Annex E
(informative)

Development and qualification of the routine test pack used for ethylene oxide sterilization

E.1 Introduction

The biological-indicator test packs for ethylene oxide sterilization referenced in 6.2.3.1 and 6.2.3.2 were originally recommended in *Good hospital practice—Performance evaluation of ethylene oxide sterilizers—Ethylene oxide test packs* (AAMI,1985). These packs were designed based on the scientific experience and professional judgment of the members of the AAMI Ethylene Oxide Sterilization Hospital Practices Working Group.

In the course of preparing a revised and expanded edition of AAMI (1985), the Working Group decided to sponsor a round-robin study to evaluate the resistance of the test pack recommended for routine monitoring of EO sterilizer performance (6.2.5.2). Annex E describes the methods used in the round-robin study and the test results. This work was also reported by Hart, et al. (1993).

Because of its make-up, the challenge test pack discussed in 6.2.3.1 offers substantially more resistance than the routine test pack. However, the resistance of the challenge test pack has not been quantified.

E.2 Materials and methods

E.2.1 Test strategy

The general test strategy of the round-robin study was to compare the resistance of the routine test pack containing a biological indicator to that of a biological indicator *not* contained within a test pack. Three types of self-contained biological indicators (Assert™ Biological Indicator No. 001500, Attest™ Biological Indicator No. 1264, and Proof Plus™ Biological/Chemical Indicator No. NA 052) and one type of spore strip (Castle® Tec-Test Biological Culturing System) were studied. All laboratories used biological indicators from the same lots.

E.2.2 Test laboratories

Five laboratories participated in the study: American Sterilizer Company, MDT Corporation, Sterilization Technical Services, 3M Health Care, and Weck Instruments.

E.2.3 Sterilization equipment

All laboratories used BIER (biological indicator-evaluator resistometer) EO exposure vessels complying with AAMI (1982) and providing the following constant sterilization cycle parameters: 600 mg/L ± 30 mg/L EO, 54 °C ± 1 °C, 60 % ± 10 % relative humidity. (A BIER EO gas vessel is a test chamber that, unlike a commercial sterilizer, allows control and monitoring of all critical cycle parameters during the exposure phase: gas concentration, temperature, relative humidity, and time.)

E.2.4 Test pack components and assembly

Each test pack used in the study consisted of a 20 ml plastic syringe with diaphragm and plunger (but no needle or needle guard), a 7 inch × 13 inch paper/film pouch, one 100 % cotton surgical towel (18 inches ± 1 inch × 30 inches ± 1 inch), and two biological indicators (one placed inside the syringe and the other attached with EO indicator tape to the outside of the test pack). Prior to assembly, the test pack components were preconditioned at 18 °C to 24 °C (65 °F to 75 °F) and at a relative humidity of 60 % ± 15 % for 2 hours to 24 hours.

The test packs were assembled in accordance with 6.2.3.2. One biological indicator was placed inside the syringe, and the syringe was placed in the center of the folds of the surgical towel. (The Assert and Proof Plus biological indicators were oriented so that their caps were next to the tip of the syringe. The spore strips in glassine envelopes were placed in the syringe.) The other biological indicator was attached with EO indicator tape to the upper corner of the test pack closest to the tip of the syringe, which was pointed toward the rear of the BIER vessel.

E.2.5 Exposure conditions

Each test cycle was run in the following manner:

a) A prevacuum was drawn to evacuate the vessel to 1 psia (pounds per square inch absolute).

b) The load was prehumidified to 60 % ± 10 % relative humidity for 30 minutes. The vacuum was increased to 2.11 psia to 2.55 psia.

c) The vessel was operated at 54 °C ± 1 °C.

d) The chamber fan was turned off during the cycle.

e) The chamber was charged with 12/88 sterilant by increasing the pressure differential by 19.7 psia ± 1 psia to provide a gas concentration of 600 mg/L ± 30 mg/L EO.

f) Each cycle was replicated three times for each of the following exposure times: 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 70 minutes, and 80 minutes.

g) Six postvacuum pulses or a 5 minute to 10 minute postcycle vacuum were drawn.

E.2.6 Test procedure

The test procedure was as follows:

a) The test pack materials were preconditioned and assembled in accordance with E.2.5.

b) A "dummy" cycle was run as per E.2.5 except for a 5 minute prehumidification time and a 5 minute exposure time.

c) The chamber was loaded with four test packs, each containing a different type of biological indicator. The packs were positioned vertically, with the outside-of-test-pack biological indicators located at the upper rear of the vessel. The pouch surfaces were oriented paper to paper.

d) At the end of the cycle, the test packs were removed from the chamber and placed in a chemical or laminar-flow hood. The biological indicators were immediately removed from the test packs and aerated at room temperature for 30 minutes to 45 minutes.

e) Within 2 hours of the end of the aeration cycle, the biological indicators were activated and cultured in accordance with the manufacturers' instructions at 36 °C ± 1 °C. The self-contained biological indicators were incubated for 48 hours; the spore strips for 5 days.

E.2.7 Data collected

For each of the seven test cycles (each of which was replicated three times), the number of surviving biological indicators (positives) and the number of killed biological indicators (negatives) were recorded.

E.3 Results

The results of the study, summarized in Table E.1, show the mean kill time for each type of biological indicator according to whether it was inside the test pack or outside the test pack. Statistical analysis of this data revealed that the mean kill time for the biological indicators inside the test pack was significantly greater than for biological indicators outside the test pack, demonstrating that the test pack indeed offers substantial resistance to the sterilization process.

Table E.1—Mean kill time (minutes) and standard deviation for biological indicators inside the test pack vs. outside the test pack

Biological indicator	Outside test pack	Inside test pack
1	19.0 ± 4.0	31.3 ± 9.7
2	26.7 ± 5.5	43.3 ± 9.9
3	26.0 ± 5.6	42.3 ± 9.0
4	26.7 ± 5.5	49.3 ± 11.4
Overall	24.6 ± 6.1	41.6 ± 11.9

Annex F
(informative)

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