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Technical Information Report

AAMI TIR16:2000

Process development and performance qualification for ethylene oxide sterilization—Microbiological aspects

AAMI
Association for the Advancement of Medical Instrumentation

Process development and performance qualification for ethylene oxide sterilization—Microbiological aspects

Approved 13 March 2000 by
Association for the Advancement of Medical Instrumentation

Abstract: This AAMI technical information report presents the various microbiological aspects of the development of an ethylene oxide sterilization process and the validation of this process. This document does not discuss the effect of the microbiological bioburden or the effect of the environment that the product is exposed to during the manufacturing process. TIR16:2000 is a companion document to ANSI/AAMI/ISO 11135:1994.

Keywords: sterilization, microbiological aspects, validation, ethylene oxide, EO, bioburden

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Association for the Advancement of Medical Instrumentation

AAMI Sterilization Standards Committee

This technical information report was developed and balloted by the AAMI Industrial Ethylene Oxide Sterilization Working Group under the auspices of the AAMI Sterilization Standards Committee. Committee approval of the TIR does not necessarily imply that all committee members and 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.

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Foreword

This technical information report (TIR) was developed by the AAMI Industrial Ethylene Oxide Sterilization Working Group under the auspices of the AAMI Sterilization Standards Committee. Ethylene oxide (EO) sterilization is an essential process that is used to render many products safe for use in health care facilities and applicable manufacturing processes. This TIR was written to provide guidance originally included in ANSI/AAMI ST27:1988 (AAMI 1988), which was subsequently superseded by ANSI/AAMI/ISO 11135:1994 (AAMI 1994). In addition, it covers recommended practices currently used by industry to terminally sterilize medical products with EO.

NOTE—Further guidance can be found in ANSI/AAMI/ISO 11737-1:1995 (AAMI 1995b) and ANSI/AAMI/ISO 11737-2:1998 (AAMI 1998).

Proper design of an EO sterilization process is based on sound scientific principles, as outlined in ANSI/AAMI/ISO standards. The cycle parameters must be validated (taking into consideration the complexity of the equipment, process, and product variables) to ensure that they are effective and reproducible. The elements of EO cycle development and validation, as defined in ANSI/AAMI/ISO 11135:1994 (AAMI 1994), should be carried out according to a written protocol or procedure with input obtained from engineering, quality/sterility assurance, and research and development (R&D) personnel.

NOTE—This technical information report is considered “informative,” and use of the terms “shall,” “should,” etc. should be considered within the context of this TIR only. That is, if the decision is made to use a particular method presented in this TIR, then the method should be followed in adherence with the requirements (“shall”) and recommendations (“should”) as set forth in this TIR. The term “must” refers to regulatory requirements.

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

Process development and performance qualification for ethylene oxide sterilization—Microbiological aspects

1 Scope

This technical information report (TIR) addresses various microbiological aspects of the development and validation of an ethylene oxide (EO) sterilization process. It does not cover the various factors that can have an effect on the bioburden of the product and on the sterilization process, nor does it specifically address parametric release. This TIR provides additional guidance to augment ANSI/AAMI/ISO 11135:1994, *Medical devices—Validation and routine control of ethylene oxide sterilization* (AAMI 1994) for medical device manufacturers, including those that use contract sterilization facilities or contract sterilization operations.

NOTE—If parametric release is being considered for the release of the product, the requirements in ANSI/AAMI/ISO 11135:1994 (AAMI 1994) for temperature parameters and monitoring should be followed. An AAMI TIR addressing parametric release is in preparation.

Although the information presented was developed for application to medical devices, the content of this guideline may also be applied to other relevant products or materials.

2 Terms and definitions

For the purposes of this TIR, the following terms, definitions, and abbreviations apply:

2.1 bioburden: Populations of viable microorganisms on a product unit.

2.2 biological indicator (BI): Inoculated carrier that is contained within its primary package and that provides a known resistance to the relevant sterilization process.

2.3 compromised tissue: Skin or mucous membrane that has been intentionally or accidentally opened, exposed, or breached.

2.4 dunnage: Material that exhibits density, temperature, humidity, and EO absorption characteristics similar to those of the actual product load.

2.5 fractional cycle: Sterilization cycle in which exposure to the sterilizing agent is abbreviated.

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

2.7 installation qualification (IQ): Obtaining and documenting evidence that equipment has been provided and installed in accordance with its specification.

2.8 operational qualification (OQ): Obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures.

2.9 performance qualification (PQ): Obtaining and documenting evidence that the equipment, as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and thereby yields product which meets specifications.

2.10 process challenge device (PCD): Object that simulates the worst case of conditions as they are given for the sterilizing agent(s) in the items of the goods to be sterilized.

NOTE 1—The design of the process challenge device depends on the kind of goods to be sterilized and the sterilization procedure. The device should be so constituted that a biological indicator can be arranged in the place most difficult for the sterilant to reach. The biological indicator should not interfere with the function of the process challenge device.

NOTE 2—In some process challenge devices, an inoculated carrier may be used in place of a biological indicator.

2.11 process development: Documented program of studies that are performed in order to define the sterilization process based upon the product/packaging/loading pattern and/or equipment limitations (ANSI/AAMI/ISO 11135:1994; ANSI/AAMI/ISO 14161:2000).

2.12 product families: Collection of products that are determined to be similar or equivalent for validation purposes.

2.13 spore log reduction (SLR): The lethality observed in a full or fractional sterilization cycle.

NOTE—SLR can be calculated as the log of the initial population minus the log of the final population. See below:

$$SLR = \log N_0 - \log N_f$$

where

N_f = final population

and

N_0 = initial population

If there are no survivors, the true SLR cannot be calculated. If one positive is assumed for purposes of calculation, the SLR should be reported as "greater than."

2.14 sterility: State of being free from viable microorganisms.

2.15 sterility assurance level (SAL): Probability of a viable microorganisms being present on a product unit after sterilization.

NOTE—SAL is normally expressed as 10^{-n}.

2.16 sterilization specialist: Person knowledgeable, by training and experience, in the science of sterilization.

2.17 validation: Documented procedure for obtaining, recording, and interpreting the results that are required to show that a process will consistently yield a product complying with predetermined specifications.

2.18 zero exposure cycle: Exposure of a validation load to all aspects of the cycle except exposure dwell.

2.19 zero exposure time: Validation cycle that consists of all aspects of the cycle except for exposure dwell, i.e. cycle in which EO is injected to the desired concentration and immediately evacuated.

3 Sterilization process development

3.1 Sterilization equipment

Guidelines for equipment selection can be found in AAMI TIR15:1997, *Ethylene oxide sterilization equipment, process considerations, and pertinent calculations* (AAMI 1997b). Careful selection of the sterilizing equipment and development of the facility design will enable a manufacturer to process a product safely and effectively.

3.2 Cycle development—Physical parameters

3.2.1 Introduction

This section discusses variables that have a significant effect during the sterilization of products: ethylene oxide (EO) concentration, relative humidity (RH), temperature, and EO exposure time.

EO concentration and RH are calculated as prescribed in AAMI TIR15:1997 (AAMI 1997b). It is recommended that vacuum and gas rates are defined initially to minimize their effect on the cycle lethality throughout the program. These rates should be incorporated in the final process specifications as appropriate for chamber parameters. It is also important to remember that the actual depth and rate of evacuations might be different for the air-removal versus the sterilant-removal phases because the product and packaging have been exposed to increased temperature, humidity, and sterilant levels prior to the sterilant-removal phase.

3.2.2 EO concentration

Common practice is to develop and validate cycles using an EO concentration ranging from 400 to 650 milligrams per liter (mg/L). It is acceptable to use lower EO concentrations, provided that the sterilization validation acceptance criteria are met. When EO concentrations higher than 650 mg/L are used, the possible increase in EO residuals should be considered.

It is important to ensure that the EO concentration can be defined for the exposure period(s) as determined during the cycle development. Depending upon device and packaging materials and design, a significant amount of EO can be selectively (as opposed to any other gas) adsorbed or absorbed by the load. This can result in a decrease of the EO concentration in the chamber. If the original chamber pressure is desired throughout exposure, the chamber pressure can be maintained by use of either EO or inert gas makeups or additions. If, because of gas absorption, an inert gas is used to maintain the chamber pressure, it is important to consider the reduction of EO concentration in the chamber over time. In mixed sterilant gas processes, the makeup gas will contain only a fraction of the EO absorbed, and the EO concentration will also decrease. In both cases, calculation of final EO concentration by chamber pressure will be difficult because the product materials will differentially absorb the carrier and EO gases.

It is recommended that the gas concentration range used in cycle development should be used for validation of the process. If the validation is successful, this range should be specified as the minimum range for routine sterilization.

3.2.3 Relative humidity

Relative humidity that is within the chamber and is in excess of 30 % is commonly used to help moisture adsorption on difficult-to-sterilize regions of the product. If a cycle has a low relative humidity during the sterilization process, the biological indicators may be inadequately humidified and, as a result, may require a sterilization process that is more severe.

Upper limits for RH should also be established during cycle development because excessive moisture could compromise the product, the packaging, or both, and could adversely affect EO transfer to product surfaces.

NOTE—The use of nitrogen and other dry inert gases during the initial vacuum and conditioning could desiccate the product, the packaging, and the microorganisms.

3.2.4 Temperature

The temperature range that is within the chamber and is used in cycle development should be used during process validation. It is recommended that this temperature range should be specified as the minimum range for routine sterilization.

During the performance qualification (PQ), the product temperature should be measured and compared with the chamber control temperature to verify that the specified product temperature can be reproduced during the exposure phase in that cycle. It is common practice to specify that the variation in product temperature during any part of the exposure phase of the cycle should not exceed 10 ^{o}C (18 ^{o}F).

If a preconditioning process is conducted outside the chamber, the PQ and subsequent requalification studies should reflect the minimum preconditioning process time, the longest chamber loading time, and the cycle start time. If preconditioning is not conducted outside the chamber, then in-chamber heating, humidification, or both should achieve minimum product temperature before the gas exposure time is initiated.

3.2.5 EO exposure time

The developmental process should set the gas exposure time requirements that are necessary to provide the desired SAL. Exposure should not be initiated or continued if the minimum specified process parameters are not met. Time specification limits should be set with reasonable tolerances to provide for operational limitations.

3.3 Cycle development—Microbiological considerations

3.3.1 Microbiological challenge systems

Microbiological challenge systems, or process challenge devices (PCDs), can be used to evaluate the delivered lethality of the selected process parameters for the product and package inside the cases during validation. They can also be used to monitor the routine process either inside or outside the case. PCDs usually consist of a known spore population of *Bacillus subtilis* subspecies *niger* or another strain of organism, known to have a resistance to EO, that has been demonstrated to be equivalent to or greater than that of *B. subtilis* subspecies *niger*.

NOTE—In the design of the PCD, consideration should be given to how the placement of the inoculated carrier could enhance or inhibit the penetration of EO or humidity.

3.3.1.1 Types of PCDs

3.3.1.1.1 Examples of systems listed below can qualify as PCDs

a) *Inoculated product*. The actual product can be inoculated directly or indirectly with spores of a known population and resistance as identified above. Direct inoculation is accomplished by applying a liquid

suspension on the product; indirect inoculation, by placing an inoculated carrier either within the package or in (or on) the product.

b) *Inoculated simulated product.* A simulated product consists of portions of a device or a combination of components that are known to represent the greatest challenge to the process while still adequately representing all products within a product family. The simulated product can be inoculated by direct or indirect means as identified in 3.3.1.1.1(a).

NOTE—Direct inoculation of a product can result in variable resistance to the inoculate because of surface phenomena, other environmental factors, and the occlusion of the spores on or in the product. Therefore, it is important to validate this practice to ensure that the resistance of the inoculated simulated product is reasonably correlated to the natural product. The inoculum recovery must also be validated if resistance is measured by plate count techniques. See Gillis and Schmidt (1983), West (1977), and ANSI/AAMI/ISO 11737-1:1995 (AAMI 1995b) for additional information.

c) *Inoculated carrier*. A carrier such as a paper strip, disc, or other substrate can be inoculated with spores of a known population and resistance. The resistance of the inoculated carrier should be correlated with the resistance of the inoculated product, simulated product, or natural product to use the inoculated carrier for cycle development (see 3.3.1.1.1[d]).

d) *Natural product*. A product with naturally occurring bioburden can be used as the microbiological challenge system for the absolute bioburden method of cycle development. When a natural product is used, it is necessary to validate the bioburden recovery techniques to establish the variability and the numbers of microorganisms per device. This method is recommended when the resistance of the naturally occurring bioburden is greater than that of the BI.

3.3.1.1.2 Examples of PCDs

Examples of PCDs include, but are not limited to:

a) an inoculated carrier placed between the rings, lands, grommets, or ribs of a syringe stopper;

b) a microbiological challenge placed in the middle of the lumen of a tube that is then reconnected using a solvent bond agent or a connector to restore product integrity;

c) a microbiological challenge placed in a stopcock interface; and

d) natural and high-bioburden material, such as unbleached cotton fabrics.

3.3.1.2 PCDs for product families

If desired, a PCD can be designed to represent an entire product family or group of families. This subject will not be discussed here. However, some guidance concerning product grouping can be obtained in AAMI/ISO TIR15843:2000*, Sterilization of health care products—Radiation sterilization—Product families, sampling plans for verification dose experiments and sterilization dose audits*, *and frequency of sterilization dose audits* (AAMI 2000a), and in a planned AAMI TIR on product family groupings for EO sterilization. Some examples of PCDs for this application follow:

a) Within a product family composed of various-sized syringes, the syringe that has been determined to represent a “worst-case challenge” could be selected as the PCD.

b) Within a product family composed of tubing sets of various lengths, the tubing set with the longest tubing could be selected as the PCD.

c) When processing kit families, the component or product that is the most difficult to sterilize in the kit should be selected to monitor the sterilization process. For example, a suction catheter kit may have a catheter, gauze pad, alcohol wipe, and a pair of latex gloves. The inoculated carrier should be placed either inside the finger of one of the gloves or inside the catheter, whichever poses the greater challenge.

NOTE—When a sterilizer load consists of more than one product family, the most resistant PCD should be used to monitor the sterilization process.

3.3.1.3 Placement of PCDs

An external PCD can be designed to validate and monitor sterilization processes. For ease of sample retrieval on routine processing, one approach consists of placing external PCDs within the sterilization load at defined sites. Because external PCDs are surrogates for internal PCDs, it is important to validate the external PCD performance against the internal PCDs by concurrent exposure during fractional or half cycles. It is preferable to compare the performance of both internal and external PCDs in the production vessel to approximate the actual conditions during routine sterilization.

If the external PCDs have a higher resistance than the internal PCDs, it might not be necessary to demonstrate total kill of the external PCDs during the half-cycle validation studies. This approach is especially valid if studies show that the internal PCDs were placed at the "most-difficult-to-sterilize" sites and that no internal PCD survivors were detected. If total kill is required for the more resistant external PCD during the validation process, the resulting cycle exposure time will provide an additional safety factor beyond the minimum requirements (see Figures 1, 2, and 3).

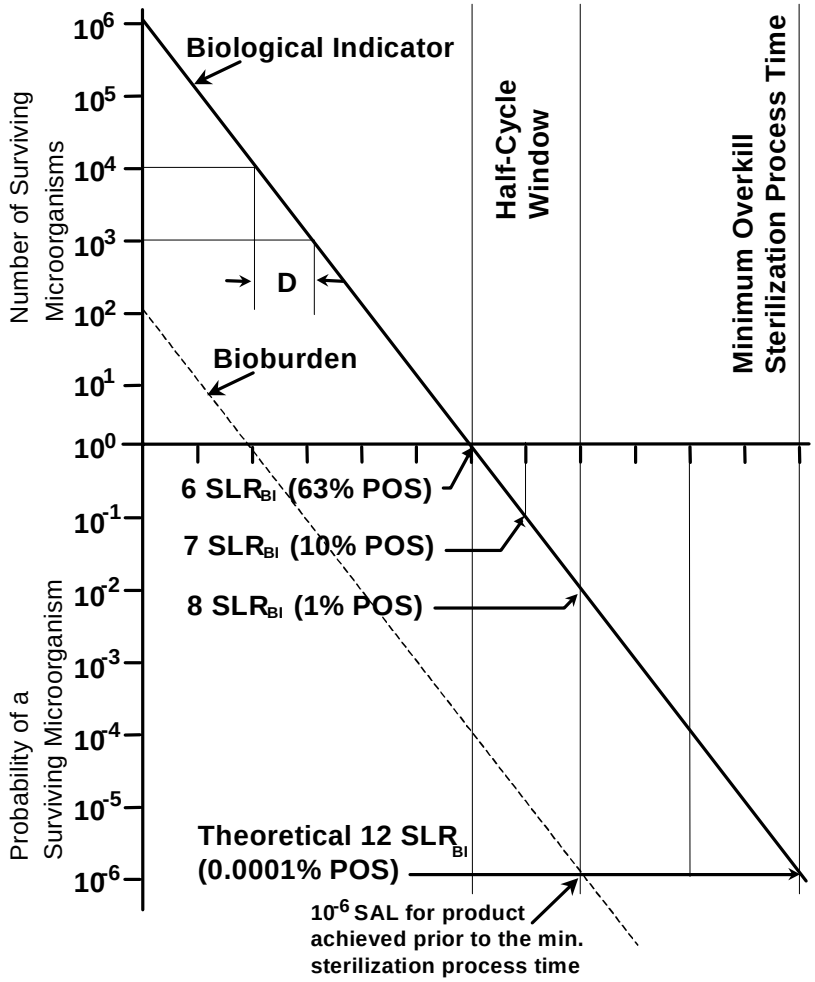

Figure 1—Example of overkill cycle

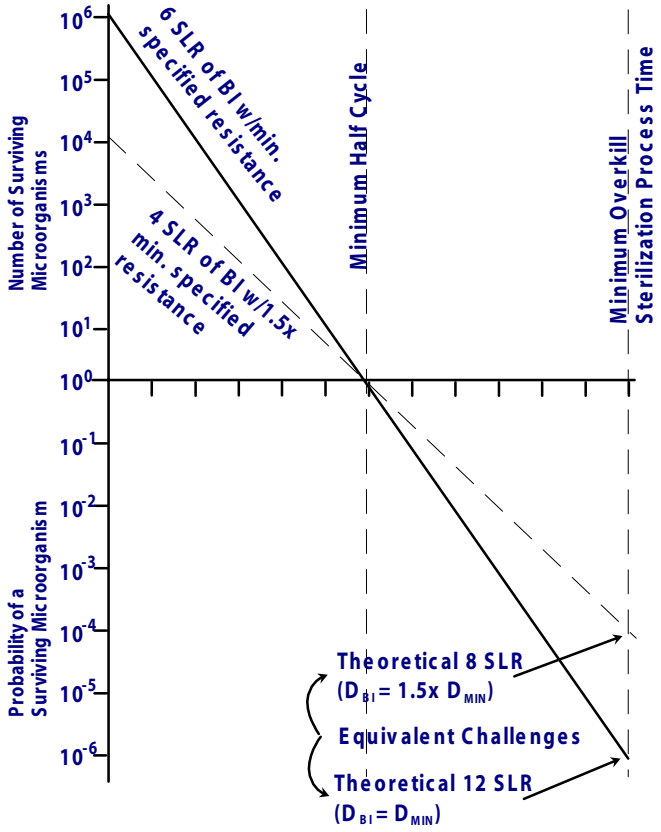

Figure 2—Examples of equivalent biological-indicator challenges for the overkill method

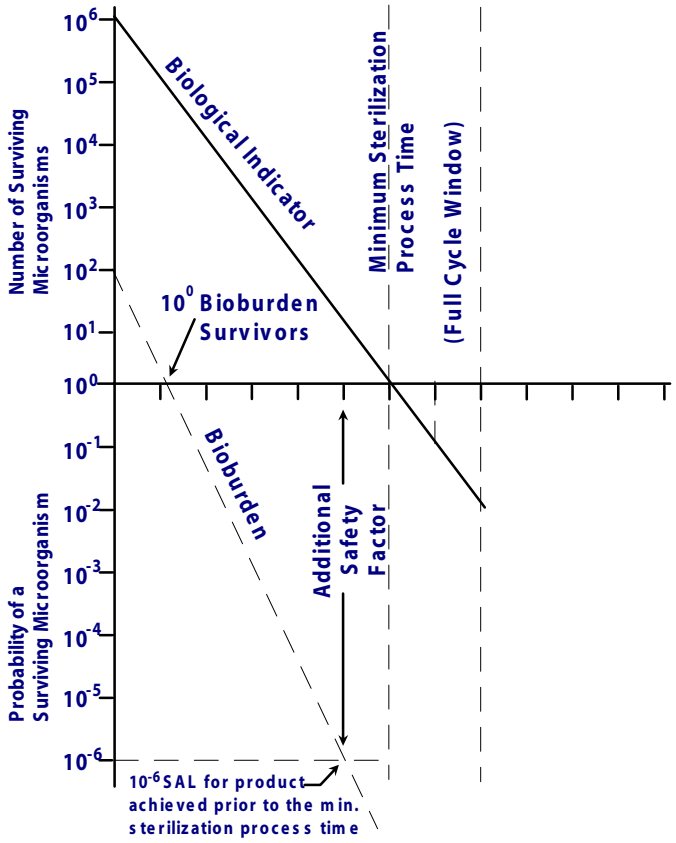

Figure 3—Example of relationship between the biological indicator and the product bioburden using the combined method

Some examples of external PCDs that have been used throughout the industry are:

a) BIs placed in plastic bags that are then placed within manila folders;

b) BIs placed in thick plastic bags that are folded over a specified number of times;

c) BIs placed in different sections of a syringe, such as inside the grommet rings or lands of the stopper; and

d) sealed plastic tubes containing an inoculated carrier, with or without additional packaging.

NOTE—If the packaging of the BI is changed by the user, the effect on the resistance of the BI should be reevaluated. Please see ANSI/AAMI/ISO 14161:2000 for additional guidance.

3.3.1.4 Appropriateness of the PCD

After the PCD has been selected, the appropriateness of its selection should be demonstrated by means of fractional-cycle studies showing that the resistance of the PCD is equal to or greater than that of the natural product bioburden. These studies are especially important if the bioburden is greater than 100 organisms (ANSI/AAMI/ISO 11135:1994, 5.4.2 and B.4), if the product is manufactured from natural fibers and materials, or if a wet process is involved. Further guidance can be found in ANSI/AAMI ST59:1999, *Sterilization of health care products—Biological indicators—Part 1: General* (AAMI 1999) and ANSI/AAMI ST21:1999, *Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization* (AAMI 1999).

If the studies indicate that the product bioburden resistance exceeds that of the anticipated PCD, one of the following approaches can be chosen:

a) A new PCD having a resistance equal to or exceeding the product bioburden resistance can be developed.

b) The product can be pretreated before sterilization to reduce the bioburden numbers.

c) The product, the process, or both can be evaluated to determine how to reduce the bioburden number or resistance (e.g., by changing the raw materials or manufacturing process used, by improving the manufacturing environment, or by modifying the product design).

d) Validation can be performed using the absolute bioburden method.

3.3.2 Microbiological challenge procedures

Common approaches used to develop effective EO sterilization cycles include the *overkill*, *combined biological indicator/bioburden*, and *absolute bioburden* methods. Subclause 3.3.3 discusses these techniques and summarizes the EO cycle selection criteria for each method, according to the product's intended use and label claims.

The PCDs described in 3.3.1.1.1(a), (b), and (c) can be used for the overkill and the combined biological indicator and bioburden methods of cycle development, while the PCD described in 3.3.1.1.1(d) is used for the absolute bioburden method and consists of natural product.

Product design and packaging can affect the rate of microbiological lethality. Therefore, studies during cycle development and validation should include a product that is assembled and packaged by standard manufacturing processes.

Lethality can be measured by recovering and counting organisms to develop a microbiological inactivation curve. A second method involves end point analysis using tests of sterility (Pflug 1990). These methods are discussed in 3.4.1.

Small research vessels provide the following features during cycle development:

— Rapid gas injections

— Quick evacuations

— Ease in test sample retrieval

If a large chamber is the only equipment available to obtain this data and the zero exposure time does not yield survivors, then different cycle parameters should be considered.

3.3.3 Development studies using microbiological challenges

3.3.3.1 Overkill method of cycle development

Due to its relative ease of use and the sterility assurance level (SAL) obtained, the overkill method is commonly used by medical device manufacturers. In this model, a 6 SLR of a 10^6 BI at half-cycle exposure time is demonstrated. This process theoretically results in 10^0 survivors. When exposure time is doubled, a 12 SLR (or a 12D process) is delivered during EO exposure. Alternatively, to reach a 10^{-2} survival or an 8 SLR of a 10^6 BI at a two-thirds cycle, exposure time requires the manufacturer to add 50% more exposure time to achieve a 12 SLR or 12D process. An overkill cycle must be adequate to provide a 10^{-6} SAL for a product with a theoretical bioburden of 10^6 EO resistant spores. See Figure 1.

Another method would be to use a biological indicator that has a greater resistance than required with a smaller population to achieve the same result. See Figure 2.

When the overkill method is used, the manufacturer should demonstrate that the resistance of the microbiological challenge test system is greater than or equal to that of the product bioburden. Fractional studies to obtain comparative resistance data do not have to be performed as frequently or as extensively as when the combined biological indicator/bioburden or absolute bioburden method of cycle development is used, but it is important to ensure that the targeted SAL can be delivered by the cycle.

Changes in product components, packaging, manufacturing methods, or environment should all be evaluated for their effect on product bioburden and its resistance. ANSI/AAMI/ISO 11737-1:1995 (AAMI 1995b) provides additional information concerning bioburden testing.

3.3.3.2 Combined biological indicator/bioburden method of cycle development

If the product bioburden is routinely tested and the population is low, then a combined biological indicator/bioburden method can be used for cycle development. This method is based on the assumption that the bioburden could be as resistant as the biological indicator (*B. subtilis* subspecies *niger* spores). An 8 SLR can be demonstrated by total inactivation of at least 100 BIs in a full sterilization cycle, or it can be predicted from survivor curve data.

If the production sterilization cycle is performed at the same parameters and with the same number of BIs, 1 out of 100 BIs could produce growth. This positive BI is likely to adversely affect product release from sterilization. Usually, however, the parameters used for routine sterilization will be greater with respect to temperature, sterilant concentration, and time. If survivor curve data is used to establish the exposure time, then the time required to achieve the maximum value (10^{-2}) of survivorship can be calculated from confidence limits. In this case, the probability of a positive BI will be significantly reduced, but it will never be zero.

An example of different combinations of bioburden population, BI population, and required SALs are shown in Table 1.

Table 1—Variations of BI-bioburden combinations for cycle development

For products requiring a 10^{-6} SAL				
Maximum bioburden	**BI population**	**# of BIs**	**SLR of BI**	**SAL of product**
$\leq$ 1,000	10^7	100	$\geq$ 9	$\leq 10^{-6}$
$\leq$ 100	10^6	100	$\geq$ 8	$\leq 10^{-6}$
$\leq$ 10	10^5	100	$\geq$ 7	$\leq 10^{-6}$
$\leq$ 1	10^4	100	$\geq$ 6	$\leq 10^{-6}$
For products requiring a 10^{-3} SAL				
Maximum bioburden	**BI population**	**# of BIs**	**SLR of BI**	**SAL of product**
$\leq$ 10,000	10^5	100	$\geq$ 7	$\leq 10^{-3}$
$\leq$ 1,000	10^4	100	$\geq$ 6	$\leq 10^{-3}$
$\leq$ 100	10^3	100	$\geq$ 5	$\leq 10^{-3}$
$\leq$ 10	10^2	100	$\geq$ 4	$\leq 10^{-3}$

As an example, if a product were to have a bioburden of up to 100 organisms, then an 8 SLR of the BI would result in an SAL of 10^{-6} (Figure 3).

3.3.3.3 Absolute bioburden method of cycle development

The absolute bioburden method is used relatively infrequently in EO sterilization because of the extensive amount of microbiological work that is required to develop and maintain the cycle. However, in two situations, this method could be the best choice:

a) when the natural product bioburden results in a higher EO resistance level than the PCD (because of a high bioburden number, a high intrinsic EO resistance, the location of the organisms on the product, or a combination of these factors); or

b) when the natural product bioburden results in a lower EO resistance level than the PCD (because of a low and relatively consistent bioburden number), and the validated cycle allows for the use of an optimized product cycle.

The absolute bioburden method involves determining the identities and resistance levels of the microorganisms that are typically found in or on the product in order to develop a cycle that will consistently deliver the desired lethality. The bioburden is screened to obtain those isolates that are most resistant to the anticipated EO process conditions. Those isolates can then be propagated and used in sterilization development studies to determine an appropriate safety factor or sterility assurance level for the product bioburden. When the natural product bioburden is used as the microbiological challenge, the inoculation level that is typically used is an average bioburden count plus three standard deviations. It is important to remember that isolation and propagation of bioburden microorganisms can alter their resistance, depending on the culturing conditions that are used.

If the information about identities and resistant levels of microorganisms is used to establish a sterilization process, then this absolute bioburden method requires environmental and process controls to maintain a consistent manufacturing process. This method also requires a validated bioburden recovery method and the performance of fractional exposure cycles on a regular basis to support the continued effectiveness of the sterilization process in accordance with written procedures. The cycle selection criteria include reduction of the challenge to a level of less

than 10^{-1}, plus an additional safety factor that depends on the product's labeling and intended use. Details of the criteria for selecting cycle exposure times are discussed in 3.4.

It is important that bioburden monitoring be frequent enough to detect changes in product components, the manufacturing environment, or production processes that could significantly affect bioburden resistance or counts. It is also important that studies include representative products from each manufacturing facility. If the monitoring program demonstrates a loss of process control through increasing counts, a documented investigation should be performed to implement a corrective action in line with the facility quality system. See ANSI/AAMI/ISO 11737-1:1995 (AAMI 1995b) for further information.

3.4 Cycle selection criteria

3.4.1 Cycle development considerations

Selection of the cycle development method is based on many factors including the nature of the product bioburden, the packaging, the manufacturing conditions, and the sterilizing equipment. The conditions required to achieve the SAL appropriate for the product can be summarized as follows:

a) Figure 1 represents an overkill cycle with the typical challenge population level of 10^6. The exposure time selected depends on the product and is derived by extrapolating the microbial inactivation curve.

b) Figure 2 compares biological indicators with different resistance levels and population counts that predict similar test results.

c) Figure 3 demonstrates how an equivalent microbiological challenge in a combined product bioburden/BI sterilization process can be achieved by reducing the biological indicator's initial population to compensate for increases in resistance.

3.4.1.1 SAL requirements

Ethylene oxide cycle exposure parameters are calculated using the cycle development information and taking into account the SAL for the product involved. The more commonly recognized SALs are:

a) SAL of 10^{-6} for products that come into contact with compromised tissue; and

b) SAL of 10^{-3} for products that do *not* come into contact with compromised tissue.

NOTE—If further information is desired on sterility assurance levels for products in the United States, please see ANSI/AAMI ST67, *Sterilization of medical devices—Requirements for products labeled 'STERILE'* (AAMI in preparation). The SAL requirement for products labeled "sterile" might be different outside the United States.

3.4.1.2 Products with more than one SAL

Some products have multiple items or components that are designed to be used for different purposes. In kits, items that are to be used on intact skin or mucous membranes or that are not intended to have patient contact might have different SAL requirements from those components that are intended to have internal tissue, neural, or blood contact. The sterilization process should deliver the required lethality to each component on the basis of the intended use of the device.

3.4.2 Methods of estimating cycle lethality

Two commonly used methods of estimating or calculating cycle lethality are described in ANSI/AAMI/ISO 11135 (AAMI 1994): the survivor curve method and the fraction-negative method. For additional information on methodology for establishing a D-value, see ANSI/AAMI/ISO 14161:2000 (AAMI 2000c), ANSI/AAMI ST59:1999 (AAMI 1999a), ANSI/AAMI/ISO 14937:2000 (AAMI 2000b), Pflug (1990), and Block (1991).

NOTE 1—BI and product sample retrieval should be performed as soon as feasible after cycle completion. Once the samples have been retrieved, the biological testing is performed following validated methods.

NOTE 2—The D-value calculations for SLRs should take into account the lethality delivered during gas injection into the chamber and postvacuum draws by minimizing the time allocated for these steps during cycle development.

3.4.2.1 Method A—Survivor curve construction

Microbiological death generally follows first-order kinetics and can be approximated by a straight line. The survivor curve can be constructed by correlation techniques using the linear regression of the logarithm of the survivors versus the equivalent gas exposure time (Figures 1 and 2). The slope of the regression line is used to estimate the SLR of the microbiological population of the PCD (i.e., the time required to reduce the microbial population by 90 %, or one log). Note that the SLR of the PCD is specific for a given set of conditions. Many variables, such as product

size and complexity, sterilizer size, and load configuration, can affect penetration of heat, humidity, and EO during routine sterilization.

3.4.2.2 Method B—Fraction-negative method

The fraction-negative method uses growth–no growth data from the tests of sterility on the PCDs after exposure to fractional gas exposure times, with all other critical parameters at minimum values. This method can be used to compare the resistance of BIs placed in multiple devices or in multiple sections of the same device. If the device size allows, graded spore strips can be used to maximize the probability of recovering survivors.

At the end of the incubation period, the number of containers exhibiting growth are counted and the number of negative (sterile) samples are determined. A graph of the PCD test results can then be used to predict the gas exposure time that is appropriate for the device, using a set of defined parameters. The user should be aware of the subsequent error that could result in the incorrect estimation of the probability of survivors.

If a specialized test vessel is used for these studies, the actual production exposure time that is necessary to achieve equivalent kill could vary because of the difference between the test vessel and the production vessel in terms of load size, pallet configuration, and density.

3.4.3 Product safety

Studies should be conducted or prior information should be provided to ensure that specified product toxicology criteria and EO residual levels are met. The primary conditions that affect aeration reduction of EO residuals are temperature, dwell time, air convection, air exchange rates, and load configuration. Safety studies should take into account the number of cycle exposures that the product may experience during the manufacturing process. Additional information on EO residuals can be obtained in ANSI/AAMI/ISO 10993-7:1995 (AAMI 1995a) and FDA (1978). Guidance on biocompatibility testing can be found in ANSI/AAMI/ISO 10993-1:1997 (AAMI 1997a).

4 Performance qualification

4.1 General considerations

A typical performance qualification requires three consecutive successful validation cycles to demonstrate reproducibility the first time the cycle is validated. The first successful cycle indicates that the proposed cycle lethality is achievable. The second successful cycle indicates that the cycle can be repeated successfully, while the third demonstrates reproducibility. If a sterilization cycle fails, it is necessary to perform a full investigation to determine the possible cause of the failure. If the failure can be attributed to a mechanical or chamber-related issue, corrective actions should be implemented and calibrations performed, if applicable, and the run repeated. If the failure was due to an inadequacy of the sterilization process, the process should be adjusted and the qualification repeated until three successful consecutive runs have been completed. See 4.4.5 for further details.

4.2 Validation cycle selection criteria

After careful review of the product design, materials, and packaging, an appropriate process is selected with parameters that have been chosen to produce microbiological inactivation without adversely affecting product or package function. If the efficacy of the sterilization cycle at the minimum specified process parameters has not been previously demonstrated, then a fractional cycle exposure performed at the minimum values will verify the effectiveness of the lower range of the established specification. If it is not possible to include presterilization and poststerilization treatments in the fractional cycle exposure, then reducing the time in the preconditioning area can provide the minimum defined load temperatures that are required by ANSI/AAMI/ISO 11135:1994 (AAMI 1994).

4.3 Placement and handling of PCDs, test samples, and sensors

4.3.1 General

A sterilization specialist or an individual who is knowledgeable in sterilization sciences should use the commissioning information and his or her professional judgment to assess the placement of samples and sensors during validation studies.

Test samples should be prepared and stored in a manner that represents the routine conditions from manufacturing to sterilization and should be placed inside product cartons or shippers. The number and distribution of PCDs should be sufficient to demonstrate the ability of the sterilization process to deliver the desired conditions for sterilization throughout the chamber load. If external PCDs have been selected for routine monitoring, they must also be placed on the product load as defined according to the protocol or procedure.

NOTE—If product bioburden resistance has been included in the development process and the resistance of the PCDs has been demonstrated to be equal to or greater than the product bioburden, then further tests of sterility on the product may not be required.

4.3.2 Cycle monitoring equipment

The installation qualification (IQ) and operational qualification (OQ) information should be used to determine placement of the temperature and relative humidity sensors so that the minimum and maximum temperature zones are incorporated into the validation. The minimum temperature zone should be evaluated for microbiological lethality and the maximum temperature zone for product and package functionality. The temperature and relative humidity sensors should be placed inside the same shippers as those containing the PCDs or as close as possible to the PCDs. If additional low temperature areas are identified during the first validation study, these sites may be incorporated into subsequent runs.

NOTE—When product temperature probing is not specified for routine sterilization, a correlation of the minimum and maximum temperature zones to the routine monitoring positions should be determined using the empty and loaded chamber profiles that are generated during the validation process.

When the sterilization of the product is performed at locations different from the manufacturing site, production-to-sterilization time frames, temperature, humidity, storage location, and other factors that might influence the sterilization process should be considered during the validation process.

4.3.3 Product handling, shipping, and testing

A procedure should be established to ensure that, at the completion of the sterilization cycle, the PCD is not left in the load longer than the proposed minimum routine aeration time that is consistent with worker protection requirements. This precaution is taken because the recovery of EO-treated spores could decrease based on the time that elapses between the end of the sterilization cycle and the test of sterility. If, however, the aeration phase of the cycle has been demonstrated to provide a part of the spore log reduction, it is necessary to include aeration time in the specification during the final determination of the sterilization process.

Shipping services should be established to allow the transfer of samples to the lab as soon as is practical after processing (preferably within 48 hours). Instructions for the shipment of samples should be clearly outlined in the validation protocol and the postprocessing instructions. If the testing of the samples is not identified in a standard operating procedure, this should also be identified in the protocol.

When the laboratory receives the shipment, the product and BIs should be placed into growth media and incubated at temperatures and times that are in accordance with the manufacturer's recommendations unless an alternate methodology has been validated.

If a product positive occurs during the testing, a failure investigation should be performed. Items to be considered are identified in 4.4.5. If no cause can be assigned, it will be necessary to reevaluate the validation cycle parameters and modify them appropriately. If an assignable cause has been identified, the product test can be repeated using samples from the load that was exposed to the validation cycle, in compliance with protocol or internal quality systems requirements. Alternatively, if additional samples from the specific validation cycle are not available, then the cycle may be repeated using new test samples.

NOTE—During the validation, the effect of product absorption of EO on the sterility test samples should be evaluated and documented by performing a bacteriostasis-fungistasis test.

All appropriate test sample controls should be included in the validation study. Positive controls should remain as part of the load to be processed until they are removed before the sterilization cycle.

4.4 Sterilization load

4.4.1 General considerations

The sterilization load that is used in the microbiological performance qualification studies should be representative of the product to be sterilized. A representative load may consist of an actual product or, if documented to be appropriate, of dunnage or a simulated product.

4.4.2 Full and partial loads

If only full loads will be sterilized in the chamber to be qualified, then a full sterilization load should be used in the microbiological performance qualification program. If smaller product loads are to be sterilized, the effect of the smaller loads on the microbiological qualification should be considered and the worst-case loading configuration should be determined because gas stratification and temperature differentials may occur. One or more of the following approaches can be taken to make this determination:

a) The worst-case loading configuration can be selected according to previously documented studies.

b) The worst-case loading configuration can be determined according to the technical judgment of a sterilization specialist. (The rationale should be documented.) In this case, it is recommended that at least one additional study be performed at the opposite extreme of loading conditions to confirm the appropriateness of the decision.

c) When there is no way to identify the worst-case loading in advance, it is recommended that a minimum of three studies be performed at the maximum specified loading configuration. An additional cycle at the minimum loading should be performed to verify that this approval does not affect process lethality.

4.4.3 Mixed loads

When performance qualification is conducted for a family of products that can have different loading configurations, the effect of the loading differences should be considered in the performance qualification program. Differences in loading configuration include, but are not limited to, the number of cartons placed onto a pallet, the arrangement of cartons on a pallet, the density of the product that is loaded onto the pallet, and shrink-wrapping of cartons on a pallet. Such differences in loading configuration can be addressed in the microbiological performance qualification program in several ways:

a) The worst-case loading configuration across the family of products can be selected according to previously documented studies.

b) The worst-case loading configuration can be determined according to the technical judgment of a sterilization professional. (The rationale should be documented.)

c) When there is no way to identify the worst-case loading in advance, it is recommended that all loading configurations be represented in the microbiological performance qualification studies.

4.4.4 Reuse of loads

When sterilization loads (products, dunnage, or simulated products) are to be reused for microbiological performance qualification studies, it is important that they be adequately aerated. The aeration period can be considered adequate if there is no residue-induced inactivation when placing biological indicators within the load and subjecting the load to the preconditioning phases, conditioning phases, or both. If preconditioning is not used, then other methods may be used to validate the inactivation of the biological indicator, such as monitoring the BI for survivor counts and D-value after the product has been placed on a pallet for a defined time that is appropriate for staging or performing EO residual studies on the load.

4.4.5 Failure investigation

If, because of a biological failure, a validation study does not meet the established requirements, then a documented investigation should be performed. Some of the issues to be considered include, but are not limited to, the following:

a) Supplier material changes

- Is there a new supplier?
- Has the supplier's manufacturing process changed?
- Is there a new source of the raw material?

b) Manufacturing or process changes

- Is different process equipment being used?
- Are there different sources of utilities such as water, air, or compressed air?
- Have the manufacturing process parameters changed?

c) Raw material changes

- Have the product dimensions changed?
- Has there been a change in the adhesive or bonding agent used?

d) Changes in the manufacturing facility

- Has production been moved from one manufacturing area or building to another?
- Has the environment changed?

- Are there different utilities?
- Are there new or different personnel?

e) Product design changes

- Have the product tolerances been modified?
- Have the assembly sequences been modified?
- Are assembly procedures now manual instead of automated (or vice versa)?
- Have new device materials been implemented?

f) Same room; however, manufacturing environment changes

- Has new equipment been added?
- Has existing equipment been modified?
- Is there construction in the facility?
- Have additional shifts or people been added to the process?
- Is there more manual handling or subassembly work?
- Have preventive maintenance and disinfection practices been validated?
- Have housekeeping practices been modified?

g) Cycle anomalies

- Has the chamber been modified?
- Are preventive maintenance procedures and calibration requirements current?
- Have there been any equipment changes?
- Are there new personnel in the area? Is training current?
- Is there any construction in the area?
- Were the physical environmental conditions different?
- Have the EO and inert gas suppliers changed?
- Has the steam supply been modified?

h) Laboratory issues

- Were the conditions in the area during PCD preparation identical to previous preparations?
- Did the same employees handle the BIs and/or PCDs in the same manner?
- Were the procedures used to prepare and test the PCDs the same as specified?
- Were the BIs handled and stored differently during receipt, quality control inspection, storage, and use?
- Were new employees involved? Is training current?
- Is the preventive maintenance and calibration of the equipment throughout the laboratory current?
- Has the environment in the test area changed?

i) BI issues from the supplier

- Has the quality of the BI and the PCD packaging for the cycle changed in any way?
- Are the BIs or package materials from a different lot or supplier?

— Were the BI and PCD preparations completed in the same way?

— Were the samples stored under specified conditions before, during, and after the shipping, EO treatment, and poststerilization treatment?

— Were the BI positive and negative controls acceptable?

j) Bioburden monitoring

— Are the bioburden numbers increasing?

— Has bioburden monitoring indicated a shift in microflora?

If an assignable cause such as a human error or an equipment malfunction can be established and is found to be an isolated event, then it is acceptable to repeat the cycle. If no assignable cause can be determined or if a process failure has occurred, it is recommended that a fractional cycle be performed to verify the cycle parameters, that the cycle be adjusted, and that the validation be repeated until three consecutive successful runs have been completed.

4.5 Shipping products to be sterilized

The effect of the coldest or driest time of the year on the sterilization of the product should be considered. AAMI TIR15:1997 (AAMI 1997b) includes a full discussion of this aspect of EO sterilization and should be used as a reference.

4.6 Release of validation loads

The product load used in a validation study may be released for customer use if the following requirements have been met:

a) If applicable, the bioburden levels are within the normal limits for products of this type.

b) The validation process parameters have been successfully attained.

c) The load has been resterilized in a full cycle after the fractional gas exposure cycle(s) has been completed successfully.

d) The sterilization cycle parameters for the full sterilization cycle were acceptable.

e) The tests of sterility for the fractional and full-cycle processes have met the protocol requirements for the validation and release of the final product.

f) The product and package met all requirements for the final functional and package integrity tests after full-cycle exposure.

g) All regulatory requirements have been met.

h) The EO residual levels have not exceeded requirements after sterilization.

i) All label claims have been met.

4.7 Release of small batches or lots

If, because of small volume production or new product development, there is only enough product manufactured to complete one sterilization load, the following validation may be performed in accordance with protocol requirements:

a) Samples are selected randomly from the batch and tested for bioburden to determine the numbers of organisms on the product.

b) A documented evaluation is made of the finished product to determine if the product can be placed into an existing product family for testing. Among the issues to consider are product composition, packaging, design, bioburden levels, and pallet density. If it is not possible to place the product into an existing product family,

— the sterilization load is exposed to a fractional gas exposure cycle at the specified minimum process parameters that are estimated to provide an SAL of $< 10^{-1}$ (see 3.4);

— the PCDs and product samples are removed for tests of sterility; and

— the load is resterilized using new PCDs, plus a sterilization cycle time that is at least twice the length of the fractional cycle.

If all PCDs and product tests of sterility are negative, then the run records are acceptable for both studies, the testing for product functionality and packaging integrity has passed, the EO residuals are within limits, all testing has been completed to meet label claims, and the product load may be released on its own merit for distribution in accordance with the requirements described in 4.6 and the manufacturer's quality system.

The remaining validation requirements should be completed, and the rationale for developing the validation cycle criteria should be documented to ensure that historic information can be retrieved and used, if applicable, to support product equivalency, cycle equivalency, or both.

4.8 Revalidation

The annual revalidation includes and may consist of only a documented review of all applicable processes by a sterilization specialist to verify that nothing has changed that would affect the process. Professional judgment should be used for this revalidation, which should include, but not be limited to, verification that:

a) there have been no changes to the product design, manufacturing and packaging materials, PCDs, suppliers, manufacturing area or facility, or manufacturing process that could affect product sterility;

b) there has not been a significant increase in product bioburden or change in the bioburden characterization, which could invalidate the delivered SAL;

c) the heat distribution and chamber operation studies demonstrate no changes over the year;

d) temperature profiles and recirculation checks indicate no changes in the preconditioning chamber or room or the aeration areas since the previous year;

e) the history of the sterilization process since the last validation demonstrates repeatability;

f) the change control and preventive maintenance programs indicate that no modifications of or changes to the sterilizing equipment have been made that could affect the process;

g) there has been no change to the sterilization process that could affect product sterility;

h) biological environmental monitoring indicates no significant shifts in either type of microbiological flora or counts; and

i) the calibration program is functioning effectively.

When a revalidation study is to be performed to confirm the continued acceptance of the process, the number of PCDs may be more or less than that recommended for the original validation (see annex B.4 of ANSI/AAMI/ISO 11135:1994), provided that the process-related data that are generated are adequate to support that the sterilization equipment and process remain unchanged. It is recommended that a revalidation study be performed at least every 2 years to verify that the documented paperwork review has captured the full concerns of the sterilization process. This review should also demonstrate that the resistance of the product bioburden has not increased to a level that would invalidate the use of the PCD or compromise the SAL claim of the process. Fractional cycles in a developmental chamber may also be used to support a revalidation program.

Annex A

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