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American National Standard

ANSI/AAMI ST34:1991

Guideline for the use of ethylene oxide and steam biological indicators in industrial sterilization processes

AAMI Association for the Advancement of Medical Instrumentation

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Association for the Advancement of Medical Instrumentation
1110 N. Glebe Rd., Suite 220
Arlington, VA 22201-4795

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ST34 Use of Ethylene Oxide and Steam Biological Indicators in Industrial Sterilization

American National Standard

ANSI/AAMI ST34—1991

Guideline for the use of ethylene oxide and steam biological indicators in industrial sterilization processes

Developed by
Association for the Advancement of Medical Instrumentation

Approved 18 June 1991 by
American National Standards Institute

Abstract:

This guideline provides the essential elements for the use of biological indicators (BIs) to develop and monitor industrial steam and ethylene oxide (EO) sterilization processes. The guideline addresses sterilization equipment, BI selection, appropriate use of BIs for qualification/validation, routine monitoring, and revalidation of sterilization processes.

Committee representation

Association for the Advancement of Medical Instrumentation
Sterilization Standards Committee

This guideline was developed by the Biological Indicators Subcommittee of the AAMI Sterilization Standards Committee. Committee approval of the recommended practice does not necessarily imply that all committee and subcommittee members voted for its approval.

The AAMI Sterilization Standards Committee has the following members:

Cochairpersons: Carl W. Bruch, PhD
Virginia C. Chamberlain, PhD

Members:
Carl W. Bruch, PhD, Dellwood, MN
Virginia C. Chamberlain, PhD, Food and Drug Administration
Neal Danielson, Wesley Medical Center, Wichita, KS
Judith Dowler, Radiation and Medical Devices, Health and Welfare Canada
Frank B. Engley, Jr., PhD, University of Missouri School of Medicine, Columbia, MO
Col. Collette Keyser, RN, U.S. Army, Retired
Robert Morrissey, PhD, Johnson & Johnson
Barry F.J. Page, Health Industry Manufacturers Association
Marimargaret Reichert, RN, MA, Reichert and Associates
Janet Schultz, RN, American Sterilizer Company

James Whitbourne, Sterilization Technical Services
James Whitby, MA, MB, PhD, University of Western Ontario, London, Ontario

Biological Indicators Subcommittee

Cochairpersons: Gary S. Graham, PhD
Gordon Oxborrow

Members: Thomas Augurt, PhD, Propper Manufacturing, Inc.
David F. Bekus, Ethicon, Inc.
Virginia C. Chamberlain, PhD, Food and Drug Administration
James Gibson, Johnson & Johnson
Gary Graham, PhD, American Sterilizer Company Medical Products Division
Lois Jones, Becton Dickinson & Co.
Michael Korczynski, PhD, Abbott Labs Hospital Products Division
Patrick McCormick, PhD, MDT Corporation
Gregg Mosely, J.R. Gillis & Associates
Gordon Oxborrow, 3M Company
Valerie Rosemond, Davis and Geck
Jack Scoville, SPS Medical
James Slade, Sherwood Medical
Laurel Stroempl, North American Science Associates, Inc.
James Whitbourne, Sterilization Technical Services
William E. Young, Baxter Healthcare

Alternates: Christopher Demitrius, Food and Drug Administration
Daniel W. Janssen, 3M Medical Surgical Division
Anna McLernon, PhD, Ethicon, Inc.

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

Foreword

This standard was developed by the Biological Indicators Subcommittee of the AAMI Sterilization Standards Committee. The objectives of this standard are to provide guidance on the appropriate use of biological indicators in developing and monitoring industrial ethylene oxide and steam sterilization processes.

Although many published articles provide guidance for using biological indicators to validate sterilization processes, no single document has provided complete and comprehensive coverage of the use of biological indicators in vessel qualification, of validation and monitoring of sterilization processes, and of the interpretation of biological indicator results. This document is intended to integrate all of the components of a validated sterilization process into a comprehensive treatise for those who are doing sterilization work using biological indicators and for those who must understand the process and interpret the results. Although it will provide useful information to health care facilities, it is primarily designed for the manufacturers of medical products. Because steam and ethylene oxide are the primary sterilization processes used — aside from radiation sterilization which is covered by the AAMI recommended practice *Process Control Guidelines for Gamma Radiation Sterilization of Medical Devices — this* document has been written to cover these processes.

The concepts incorporated in this document should not be considered inflexible or static. To remain

relevant, this standard must be reviewed and updated periodically to assimilate progressive technological developments. This standard reflects the conscientious efforts of those individuals and organizations substantially concerned with its scope and provisions to develop a standard for those performance levels that can reasonably be achieved at this time.

Suggestions for improving this standard are invited. Comments should reference specific paragraph numbers, provide suggested alternative wording, and explain the rationale for each recommendation. Comments/suggested revisions should be sent to AAMI, 3330 Washington Boulevard, Suite 400, Arlington, VA 22201-4598.

NOTE — This Foreword does not contain provisions of the American National Standard *Guideline for the Use of Steam and Ethylene Oxide Biological Indicators in Industrial Sterilization Processes* (ANSI/AAMI ST34-1991), but does contain information about its development and intended use.

Guideline for the use of ethylene oxide and steam biological indicators in industrial sterilization processes

1 Scope

1.1 General

This guideline provides the essential elements for the use of biological indicators (BIs) to develop and monitor industrial steam and ethylene oxide (EO) sterilization processes. This guideline addresses sterilization equipment, BI selection, appropriate use of BIs for qualification/validation, routine monitoring, and revalidation of sterilization processes.

1.2 Inclusions

This guideline is directed to all industrial manufacturers who sterilize medical products with steam or EO and to those who perform contract sterilization.

1.3 Exclusions

1.3.1 Sterilization processes other than steam and EO are excluded from this guideline.

1.3.2 Sterilization processes in health care facilities are excluded from this guideline.

1.3.3 Dry heat sterilization is not specifically covered in this guideline. Nevertheless, the principles outlined will generally be applicable.

1.3.4 This document does not address processes based on bioburden only or parametric release.

1.3.5 Specifically excluded are those vessels that employ sterilization processes other than steam or EO, and synergistic processes employing steam or EO. Also excluded are fermentation vessels of the sterilize in place (SIP) type used in the pharmaceutical industry.

2 Normative references

The following documents contain provisions which, through reference in this text, constitute provisions of this American National Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this American National Standard are encouraged to investigate the possibility of applying the most recent editions of the documents indicated below.

2.1 *AAMI BIER/Steam Vessels,* 1st ed. Arlington, Virginia. Association for the Advancement of Medical Instrumentation, 1981. AAMI BSV.

2.2 *AAMI BIER/EO Gas Vessels,* 1st ed. Arlington, Virginia. Association for the Advancement of Medical Instrumentation, 1981. AAMI BEOV.

2.3 *AAMI Biological Indicators for Ethylene Oxide Sterilization Processes in Health Care Facilities,* 1st ed. Arlington, Virginia. Association for the Advancement of Medical Instrumentation, 1985. AAMI ST21.

2.4 *AAMI Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices,* 2nd ed. Arlington, Virginia. Association for the Advancement of Medical Instrumentation, 1988. American National Standard, ANSI/AAMI ST27.

2.5 *AAMI Guideline for Industrial Moist Heat Sterilization of Medical Products,* 1st ed. Arlington, Virginia. Association for the Advancement of Medical Instrumentation, 1987. American National Standard, ANSI/AAMI ST25.

2.6 *USP United States Pharmacopeia, USP XXII.* Easton, PA. Mack Publishing Co., 1990.

3 Definitions

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

3.1 absolute pressure: The pressure measured when the reference baseline is 0 and not atmospheric pressure. For example, gauge pressure uses atmospheric pressure as a reference point and pressures are measured relative to the atmosphere.

3.2 ambient pressure: The prevailing atmospheric pressure. Ambient pressure varies with the altitude of the location at which it is measured and with weather conditions, but is approximately 14.7 psia at sea level.

3.3 bacterial count: A method of estimating the number of bacteria per unit sample. The term also refers to the estimated number of bacteria per unit sample, usually expressed as number of colony-forming units (CFUs).

3.4 BIER/EO gas vessel: The abbreviation for biological indicator-evaluator resistometer, which is a pressure vessel that permits precise control and monitoring of ethylene oxide exposure conditions (gas concentration, temperature, relative humidity, and time) with minimal lag times between cycle phases.

3.5 BIER/steam vessel: The abbreviation for biological indicator-evaluator resistometer, which is a pressure vessel that permits precise control and monitoring of saturated steam pressure and temperature with minimal lag times between cycle phases.

3.6 ballast: Material (such as scrap material) used to stabilize a partial load so that full-size load conditions can be simulated; for example, to stabilize heat-up conditions.

3.7 bioburden: The number and types of viable microorganisms with which an item is contaminated; also known as bioload or microbial load. When measured, bioburden is expressed as the total count of bacterial and fungal colony-forming units per single item.

3.8 biological indicator (BI): A sterilization process monitoring device consisting of a standardized, viable population of microorganisms (usually bacterial spores) known to be resistant to the mode of sterilization being monitored. Biological indicators are intended to demonstrate whether or not the conditions were adequate to achieve sterilization. A negative biological indicator does not prove that all items in the load are sterile or that they were all exposed to adequate sterilization conditions.

3.9 biological indicator test unit: The form of biological indicator in its final package configuration used as a test sample in a sterilization process.

3.10 calibration: The comparison of a measurement system or device of unknown accuracy to another measurement system or device of known accuracy, the purpose of which is to detect, correlate, report, or eliminate by adjustment any variation from the required performance limits of the unverified measurement

system or device.

3.11 CFU: Colony forming unit

3.12 confidence limits: The upper and lower limit values of a range in which there is a stated probability that the true mean value is contained within that range. If the probability is 0.95 that the true mean does lie within the defined range, it can be stated with 95% confidence of being correct that the range does include the true mean. The greater the variation in the sample and the raw data used to calculate a mean value for a sample of a population, the greater the uncertainty becomes, and thus the greater the range needed to have a 95% confidence that the range includes the true mean value of a population.

3.13 count reduction: The difference between the initial number of microorganisms and the number of microorganisms surviving after a partial sterilization process.

3.14 culture medium: A substance or preparation used to grow and cultivate microorganisms.

3.15 cycle time: The total elapsed time of a sterilization cycle from the time the door is closed until the cycle is completed and the door opened, which may include heat-up time, exposure time, come-down time, cooling and/or drying time, and, on appropriate equipment, pre- and post-vacuum time.

3.16 cycle time reduction value: The time required to kill 90% of spores on a biological indicator when the biological indicator is placed in a test pack. See also **D value.**

3.17 D value: The exposure time required under a defined set of conditions to cause a 1-logarithm or 90% reduction in the population of a particular microorganism. The larger the D value, the more resistant the microorganism is to thermal destruction. The value can be derived by plotting the logarithm of the number of microbial survivors against sterilization exposure time; the time corresponding to a 1-logarithm reduction in numbers may then be directly measured.

3.18 dichotomous result: A split or divided result; some portion of the total units tested are positive for growth while the remainder of the units tested are found to be negative for growth.

3.19 dwell time: The amount of time selected on the instrument control panel for the load to come into equilibrium with the sterilizing chamber environment.

3.20 enumeration analysis: Method for determination of cycle efficacy based on enumerating survivors.

3.21 exposure period: The total elapsed time during which the sterilizer is operating at the preselected sterilizing conditions.

3.22 F value: A measure of the microbial inactivation capability of a heat sterilization process. The F value is calculated by determining the lethal rate per minute at each process temperature using the z value of the microorganism.

3.23 fraction negative or quantal assay: A mathematical method permitting the estimation of the number of surviving microorganisms after sterilization processing, when the probability of a microorganism surviving is between 1.0 and 0.01.

3.24 fraction positive: The number of positive sterility tests in relation to the total number of exposed samples.

3.25 GMP: Good Manufacturing Practices

3.26 heat shock: A screening process to isolate bioburden that exceeds a minimum heat resistance. The application of a known amount of moist heat to the bioburden allows the isolation of bioburden microorganisms having moist heat resistance. This process inactivates the less resistant vegetative bioburden microorganisms but allows the more resistant bacterial spores to recover.

3.27 hysteresis: The lag in response to any given stimulus.

3.28 installation qualification: As the term is used in industrial sterilization processing, a step in the sterilization validation program that establishes, through appropriate studies and documentation, that the process equipment can perform within its design specifications.

3.29 lot: A definite quantity of some product manufactured or sterilized under conditions considered to be uniform; also known as a batch or group.

3.30 lot control number (load control number): A combination of numbers, letters or both, by which a particular group of products may be traced to a particular manufacturing or sterilization operation.

3.31 master product: As the term is used in industrial sterilization processing, the product that demonstrates the slowest logarithmic rate for microbial reduction in a given sterilization cycle based on mean D and z values.

3.32 microbial challenge: As the term is generally used in sterilization science, a biological indicator, biological indicator test pack, or inoculated product that contains a known population of microorganisms and that is used in sterilization cycle development, validation, and routine monitoring to demonstrate the microbial lethality of the sterilization process. Strictly speaking, a microbial challenge is the microbial load (in any form) presented to a sterilization process.

3.33 microbial challenge tests: Tests that measure the microbial lethality of the sterilization process.

3.34 PSIA: Pounds per square inch absolute. Units for expression of pressure beginning at absolute zero pressure (absolute vacuum).

3.35 PNSU: Probability of a non-sterile unit

3.36 preconditioning: A step in the sterilization process prior to exposure to the sterilizing gas mixture, designed to bring the product to specified conditions of temperature and relative humidity. Preconditioning can be performed within the chamber, in an external area, or in both.

3.37 positive control: As the term is used in routine sterilization process monitoring, a biological indicator, from the same lot as a test biological indicator, that is left unexposed to the sterilization cycle and then is incubated to verify the viability of the test biological indicator.

3.38 primary package: As used in relation to biological indicators, the package that encloses the spore carrier and maintains its integrity.

3.39 SAL: Sterility assurance level. The expected probability that an item is sterile after exposure to a valid sterilization process, often expressed in terms of the probable maximum frequency of contamination.

3.40 self-contained BI: A biological indicator equipped with a source of culture media so that aseptic transfers from the BI package to a separate culture system are not required.

3.41 spore crop suspension: (microbial challenge suspension). A suspension containing spores of microorganisms of known concentration and heat resistance, which can be expected to follow a predictable death rate when exposed to a known physical or chemical condition.

3.42 sterile/sterility: The state of being free from all living microorganisms. In practice, sterility is usually described as a probability function; for example, as the probability of a surviving microorganism being one in a million.

3.43 sterilization validation: A documented program of testing to demonstrate that medical products can be sterilized reliably by the designed sterilization process, and that the process will consistently produce a product meeting predetermined specifications and quality characteristics.

3.44 synergistic process: The process of two forces, such as EO and steam, acting together to enhance the

effect of another force or agent.

3.45 temperature distribution study: A study to determine the temperature profile within a sterilizing chamber during a sterilization cycle.

3.46 validation: The establishment of documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting predetermined specification and quality attributes.

3.47 z value: The number of degrees of temperature required for a 1-logarithm change in the D value. A z value can be obtained from a thermal resistance curve; D values are plotted against temperature, and the reciprocal of the slope is determined as the z value.

4 Equipment — EO and steam

4.1 General statement

The resistance characteristics of BIs employed to monitor industrial sterilization processes should be determined in an appropriate BIER vessel constructed to AAMI specifications. The BIER vessel should incorporate adequate safety features (e.g., proper electrical grounding, safety interlocks, pressure relief valves or discs) designed to minimize potential hazards.

In addition to their primary function in determining the resistance performance characteristics of biological indicators, BIER vessels may also be employed in cycle development studies. They allow one to measure with a high degree of precision the effect on lethality of different cycle parameters, and are also useful for characterizing the effect of such factors as packaging materials, product configuration, and the substrate or material onto which the indicator organisms are inoculated.

For applications where the precise control and small chamber size of the BIER vessel do not allow for the simulation of the production chamber, intermediate size vessels, known as laboratory or pilot vessels, are often employed. These vessels allow one to model the behavior of the desired items under a variety of different process conditions without tying up production vessels. It is essential, however, that the relationship of the laboratory or pilot vessel to the production vessel be characterized. This requires consideration of factors such as the come-up and come-down times, cycle hysteresis, air removal, and stratification. Once this relationship is established, it may then be possible to extrapolate the information gained from studies in the laboratory or pilot vessel to the production vessel by applying the appropriate correction factors. As with any pressure vessel, appropriate safeguards must be incorporated into the design and operation of the vessel to minimize potential hazards to the operator.

The design of a BIER vessel provides reproducible sterilization conditions within a controlled set of parameters. The performance of the BIER vessel shall be such that it is capable of performing to those specifications set forth in the applicable AAMI BIER vessel standards (see normative references 2.1 and 2.2) and listed below (Tables 1 and 2). Documentation in the form of drawings or schematics of, for example, plumbing and wiring, should be provided by the manufacturer of the BIER vessel along with instructions for use. Evidence that the BIER vessel performs to its design specifications should be made available by the manufacturer and a record of performance maintained by the operator. This record may take the form of chart recordings, a logbook of visual observations made during use of the unit (e.g., operating temperatures and pressures) or other suitable means. Records should also be kept of the calibration of the instrumentation. This calibration should be performed using reference instruments traceable to the National Institute of Standards and Technology. Frequency of calibration should be determined by the user based on the user's experience. The procedures employed to perform calibration along with any routine maintenance or repair performed on the vessel should also be documented.

As used here, the term "industrial sterilization vessels" shall refer to those vessels employed for the routine steam or EO sterilization of products or devices intended for distribution to the health care industry. An

"industrial sterilization vessel" is one designed to provide a uniform set of sterilizing conditions within a chamber or vessel where the products or items to be sterilized are placed. Means are provided to closely monitor and control the conditions within the chamber during sterilization and to allow for access and removal of products from the chamber. The load is sterilized under conditions that have been previously validated to achieve the desired levels of sterility assurance, and the vessel is capable of reproducing these conditions. The vessel should be operated and maintained according to Good Manufacturing Practices.

Industrial sterilization processes are often designed for a particular application, and it is neither practical nor desirable to establish a given set of performance criteria for all industrial sterilization vessels. It must be demonstrated that the vessel is capable of operating within the desired parameters and that these parameters are capable of delivering the desired sterility assurance level (SAL) in a manner consistent with Good Manufacturing Practices. Calibrated BIs are an important part of the cycle development, validation, and monitoring process and may be employed to demonstrate that the sterilization process has achieved the targeted level of microbial inactivation.

NOTE — Those operating or otherwise coming into contact with either BIER test vessels, laboratory or pilot vessels, or industrial sterilization vessels should be properly trained in the operation of these vessels and in the procedures to be followed in the event of equipment failure.

4.2 BIER/steam vessels

BIER/steam vessels used to determine the resistance characteristics of BIs employed for saturated steam processes should conform to the criteria set forth in normative reference 2.1. At a minimum, the vessel should be capable of meeting the performance criteria listed below (Table 1).

Table 1 — Minimum performance criteria for BIER/steam vessels

Parameter	Criteria
time to temperature	10 seconds
time to exhaust	5 seconds
operational temperature	121 ± 0.5°C
operational pressure	29.7 psia ± 0.5

4.3 BIER/EO gas vessels

BIER/EO gas vessels used to determine the resistance characteristics of BIs employed for EO sterilization processes should conform to the criteria set forth in normative reference 2.2. At a minimum, the vessel should be capable of meeting the performance criteria listed below (Table 2).

Table 2 — Minimum performance criteria for BIER/EO gas vessels

Parameter	Criteria
time to gas concentration	not to exceed one minute
time to exhaust	not to exceed one minute
exposure conditions	600 ± 30 mg/L EO
sterilant temperature	54 ± 1°C
percent relative humidity	50-70% RH

5 Selection of biological indicator

5.1 General

A biological indicator can be defined as a unit containing microorganisms or spores of a specified concentration and resistance to a given sterilizing agent that are expected to follow a predictable death rate

when exposed to certain physical and/or chemical parameters. The unit can demonstrate that the conditions necessary for sterilization were achieved, but cannot by itself validate that product sterility has been obtained. This can only be determined as a probability function through a validation program.

The use and type of measurement obtained from the BI may vary depending upon whether one is using it for cycle development, cycle validation, or routine monitoring. In general, these types of measurements can be divided into two categories: enumeration analysis and quantal or fraction-negative analysis.

Self-contained BIs are dependent on the spores and media supplied, therefore subculturing to another medium is inappropriate. The design of some types of BIs may affect the survivor curve, particularly for short exposure times in steam sterilization.

5.1.1 Enumeration analysis

This method, in which the number of viable organisms in or on the BI is counted or enumerated, is generally referred to as the count reduction method. The surviving number of microorganisms is evaluated, using standard microbiological plating techniques, at fractional sterilization cycle exposure times (see Figure 2). In this manner, the degree of lethality as a function of proposed process parameters can be used to develop a cycle death-rate curve. This method is most frequently used during cycle development, but may also be applied for cycle validation.

5.1.2 Quantal or fraction-negative analysis

In this method the spore inoculated carrier is placed in a broth medium and evaluated for a growth/no growth response. Replicate units are exposed to the entire or to a fractional cycle, cultured, incubated, and scored for growth (positive) or no growth (negative). There are three possible outcomes: (1) all units are positive for growth, (2) all units are negative for growth, or (3) some units are positive for growth and some units are negative for growth. When outcome "3" occurs, the data can be used to estimate the number of surviving organisms per unit using the most probable number (MPN) analysis of Halvorson and Ziegler (Halvorson 1983). The quantal analysis methodology is most frequently used for routine monitoring or for cycle validation, but may also be used for cycle development. In routine monitoring, negative growth of all BIs only provides information that a certain minimal sterilization dose was delivered. See sections 5.3.3, 5.3.5., 6.5.3.3, and 6.5.3.4 for details.

5.2 Types of BIs

BIs may be available in different forms depending upon the intended usage (i.e., cycle development, cycle validation, or routine monitoring).

NOTE — Different regulatory, compendial, and guideline setting organizations may have varying requirements pertaining not only to the type, but also to the resistance performance characteristics of BIs. For additional information, consult the normative references (section 2) and annex B.

5.2.1 Paper carrier BI

Spores from an appropriate strain of bacteria are inoculated onto a paper carrier. Paper carrier monitors are prepared with spores to monitor steam sterilization or EO sterilization, although carriers prepared with two kinds of spores to monitor both types of sterilization are also available. Use of the paper carrier BI requires aseptic culturing techniques using soybean-casein digest medium or equivalent. The paper carrier BI is suitable for enumeration analysis or for fraction-negative analysis.

The results of these two types of analysis may vary because of differences in culture conditions and the effect of different brands of commercially available soybean casein digest medium (Pflug, et.al 1981).

5.2.2 Self-contained BI

A self-contained unit provides a bacteriological culture medium with the spore inoculated carrier and

eliminates the need for BI transfer to a separate culture medium.

Due to the large size of the self-contained BI, compared with the paper carrier BI, it may not be suited for placement at a difficult to sterilize area of a device. Studies may have to be performed to demonstrate its comparability to an alternate BI at a difficult to sterilize area of the device in question.

5.2.3 Sealed ampule BI

This type of indicator consists of a hermetically sealed container, usually a glass ampule, with spores of *Bacillus stearothermophilus* suspended in soybean casein digest medium or equivalent. This type of indicator is suitable for monitoring steam sterilization of liquids. It is not used for enumeration analysis and is best suited for validation or routine monitoring of a process. When using this system with a fraction-negative type analysis, aseptic culturing techniques are not required.

5.2.4 Spore suspension

Spore suspensions prepared from an appropriate microbial strain can be used to prepare inoculated product or inoculated simulated product. Inoculated product can be prepared to monitor steam or EO sterilization.

For products or materials, the use of direct inoculation with a spore suspension may cause prolonged survival of spores under normal sterilization conditions. Inoculated product or simulated product is used in enumeration or in fraction-negative analysis. This type of biological indicator requires aseptic culturing techniques, is typically used in cycle development, and may be appropriate for assessing factors such as product sterilizability, most difficult to sterilize location, and localized bioburden effects.

NOTE — BI manufacturers normally determine resistance characteristics of spore suspensions inoculated on paper carriers selected by the manufacturer and placed in glassine envelopes. Variations as to the lot number, grade, or manufacturer of the paper or glassine may affect resistance patterns. Direct inoculation of a product can result in higher or lower resistance values compared with other challenge systems (Gillis and Schmidt 1983; West 1977).

5.2.5 Correlation of resistance characteristics among BI types

Due to their design and construction, different types of BIs may provide significantly different challenges to the sterilization cycle. If the BI to be used in routine sterilization monitoring is different from that used in development studies, the routine process monitor must be demonstrated to provide a known relationship with an adequate challenge developed from the validation studies (see sections 6.1 and 9).

5.3 BI performance criteria

The performance criteria used in the selection of a BI will vary depending upon three factors: (1) whether the unit will be used for enumeration or quantal analysis, (2) whether the unit is being used for cycle development, cycle validation, or routine monitoring of manufacturing, and (3) whether the cycle is based upon the overkill method or the bioburden method. These factors must be considered in the selection and appropriate use of the biological indicator.

5.3.1 General

During sterilization cycle development, the spore challenge should be placed in the areas of the device that are most difficult to sterilize. Thus, the BI must be of a size that can be physically located at various sites within the device. The use of direct product inoculation with a spore suspension may be required. If the BI is to be located at more convenient product locations, or if a different type of BI is used during routine monitoring of manufacturing, correlation studies should be performed during cycle development and validation in order to demonstrate that the more convenient location or indicator type exhibits a known relationship with indicators placed at the most difficult to sterilize site.

The selection of the BI will depend upon the type of microbiological analysis required. The spore challenge

may be evaluated at fractional exposure times to determine the degree of lethality as a function of proposed sterilization parameters. The degree of lethality at the different fractional exposures is measured by recovering and counting surviving organisms to develop a death rate curve, or by an end-point analysis. A self-contained BI for instance, would not be appropriate if a count of surviving organisms was desirable, but it would be appropriate for an end-point analysis. On the other hand, a paper strip BI is appropriate for either type of analysis. Regardless of the method used or the type of BI chosen, the appropriate resistance performance characteristics of the BI may vary depending upon whether the sterilization cycle is based upon an overkill or a bioburden approach.

These procedures may not be applicable to parenteral solutions and their associated containers and closure systems.

5.3.2 Steam sterilization-overkill method

The overkill approach is based on the premise that the sterilization process will inactivate the spore challenge and provide an additional safety factor. For example, the overkill cycle is based on the inactivation of at least 12 logarithms of a microorganism with a $D_{121°C}$ of approximately 1 minute. Inactivation of not less than 12 logarithms of a microorganism with a $D_{121°C}$ of approximately 1 minute under the conditions of use will result in minimal product F_0 values of approximately 12 (based on a reference z value of 10°C). A cycle that demonstrates a 6-log spore reduction of a microbial challenge with a $D_{121°C}$ of approximately 1 minute, and a z value of approximately 10°C, at one-half the sterilization cycle exposure time is an example of an overkill-based cycle.

When the microbial challenge has a D value of other than 1 minute then the log of the spore population multiplied by the $D_{121°C}$ value should result in a minimum value of 6 minutes at the half cycle.

The BI should be certified by the manufacturer (see 6.3 and normative reference 2.6). The z value of the BI should be approximately 10°C. The D value and z value of the BI can vary in different environments and at different sites (e.g., closures) within the same product. The z value of a microorganism is a measure of how heat resistance changes with changes in temperature. "z" is defined as the number of degrees temperature change required to change the D value by a factor of ten.

NOTE — The z value for a BI should be verified when the indicator is used to measure integrated lethality during development and validation of a production sterilization cycle. Discrepancies may occur between F_0 values determined by thermocouple (F_0 assumes z = 10°C) and F values determined by BIs.

5.3.3 Steam sterilization — combined BI/bioburden method

This method may employ any of several types of moist heat resistant spores as BI systems. Strains of spores that demonstrate high moist heat resistance with respect to bioburden are *C. sporogenes, B. coagulans, B. subtilis* and B. *stearothermophilus.* The microbial population on the BI should be compared to the mean number plus three standard deviations of the bioburden population associated with the product; also, the sterilization resistance of the BI must be compared to the sterilization resistance of the manufactured product. The comparison should demonstrate that inactivation of a predetermined level of indicator organisms ensures a probability of survival of no less than the desired SAL.

The combined BI bioburden method requires bioburden count and identification. Representative products from routine production should be sampled. The bioburden determination should take into account any significant changes in components of the product, methods of manufacturing, environmental factors, or handling of the product. Bioburden data should be obtained to establish a historical trend. The frequency of bioburden determination depends on the variability of the historical data, the kind of products being sterilized, knowledge of the manufacturing process, and type of sterilization process. If a change in the manufacturing environment occurs, additional bioburden monitoring should be done.

The combined BI bioburden method may include the identification of thermal resistant microorganisms. For instance, bioburden isolates may be propagated and used for resistance evaluation in order to determine the more resistant bioburden isolate. The BI must be compared to the sterilization resistance of this more resistant bioburden isolate.

NOTE — The isolation and propagation of bioburden microorganisms can alter their resistance, depending on the culturing conditions used. Product and bioburden microorganisms may also interact (naturally or subsequently upon reinoculation), thereby increasing or decreasing the bioburden resistance.

A high degree of expertise is required to minimize these effects. These procedures are beyond the scope of this guideline.

5.3.4 EO sterilization-overkill method

The microbial challenge consists of selected numbers of EO resistant spores. Typically, 10^6 spores of *B. subtilis* var. *niger* are used. A cycle that demonstrates a 6-log spore reduction of the microbial challenge, at one-half the sterilization cycle EO exposure time is an example of an overkill cycle.

The D value, at exposure conditions of 600 ± 30mg EO/liter, 54 ± 1°C, and 60 ± 10% relative humidity, for the BI should be a minimum of 2.6 minutes. In addition, the population should be such that the BI demonstrates survival of spores on 100% of the strips following a minimum 10.4-minute exposure to these same test conditions in a BIER/EO gas vessel. The units should also demonstrate 100% kill when exposed to the same conditions for a maximum of 58 minutes (normative reference 2.6).

5.3.5 EO — combined BI/bioburden method

This method uses spores of *B. subtilis* var. *niger* in the BI system. The microbial population of the BI should be compared to the mean number plus three standard deviations of the bioburden population associated with the product. The sterilization resistance of the BI also must be compared to the sterilization resistance of the manufactured product. The comparison of such data should demonstrate that the inactivation of a predetermined level of BI microorganisms would assure an appropriate sterility assurance level (SAL) for the product when bioburden is considered. For instance, a cycle may be developed that would have the capability of reducing the BI to a level of probability of a survivor of 10^{-3} or less, but be capable of assuring that the probability of a bioburden survivor is 10^{-6} or less.

The combined method is used when sufficient bioburden information shows that a microbial challenge population level lower than 10^6 per carrier can be used. The resistance of the BI must be compared to the resistance of the product's bioburden. A comparison of such data must demonstrate that conditions inactivating the microbial challenge will result in equal or greater destruction of the product bioburden.

This method requires the collection of bioburden data and the correlation of numbers and/or bioburden resistance to the microbial challenge system employed. A bioburden monitoring program must be operational so that changes in product components, packaging, the manufacturing environment, or production processes that could affect bioburden, may be evaluated. Bioburden monitoring frequency depends on the consistency and reproducibility of current and historical data. If more than one manufacturing facility exists for the same product, studies should be performed using products from each facility. Different facilities may have products with different quantities and strains of microbes.

In order to correlate BI and bioburden resistance, representative product and BI units may be subjected to incremental exposures. The units can be tested to determine the number of positive samples (for both BI and bioburden) after each exposure period, or surviving microorganisms can be recovered and counted to establish a microbial inactivation curve.

As an alternative, products may be analyzed to obtain isolates resistant to the proposed EO process

conditions. The isolates can be propagated and their resistance compared to the biological indicator. To ensure reliable results, additional factors must be considered. See Section 7.4 for additional information.

6 Qualification/validation of the BI

6.1 General

For each BI system, the microbial population, the expiration date, the storage conditions, the resistance performance characteristics, the culture media and conditions for use (such as temperatures and duration of incubation) should be specified. For commercially available BIs, this information is provided in the labeling. Verification of the labeled performance characteristics of commercially purchased BIs is required. Minimal requirements for this verification would include receipt of Certificate of Performance from the vendor, and a quality assurance audit of the vendor's process and testing program.

6.2 Certificate of performance

A Certificate of Performance should be available for each lot of BIs purchased. This certificate should include the following information:

a) the BI organism(s)

b) lot number

c) viable spore population count

d) resistance performance characteristics under specified test conditions (time, temperature, RH, and gas concentration)

e) expiration date

f) recommended storage conditions

6.3 Audit of BI manufacturer

Since many manufacturers of sterile products do not have the capability of verifying BI label claims in their own laboratory, it is recommended that a quality assurance audit of the BI manufacturer be performed. Since many factors can affect the performance of biological monitors, an outline of factors to be considered in the quality assurance of BI manufacturing is given below:

A) Spore suspension

1) Strain selection and maintenance

2) Spore propagation procedures

a) Growth media and components

b) Growth temperature

c) Incubation period

3) Harvesting and cleaning procedures

a) Species

b) Microbial purity

c) Cleanliness

d) Population

4) Resistance testing

a) Equipment and calibration

b) Exposure conditions

c) Methodology (fraction-negative or enumeration)

d) Recovery media

e) Incubation temperature

f) Post-exposure incubation duration

5) Storage and maintenance

a) Stability

b) Periodic requalification

B) BI preparation

1) Component prequalification and preparation

a) Carrier material

b) Packaging material

c) Toxicity of materials

2) Inoculation

a) Population distribution

3) Assembly and packaging

a) Fill volume (self-contained)

C) Quality control

1) Verification of label claims

a) Population

b) Resistance

c) Stability

d) Shipping consideration (see note).

2) Acceptance criteria

D) Personnel qualifications and training

NOTE — The effect of shipping conditions on lot to lot variability may be assessed using sections 6.4 and 6.5.

6.4 Population verification

The precise methodology for determining the spore population of the BI will vary depending upon the type of BI and the manufacturer. If the manufacturer does not supply a recommended procedure, the following method should be used.

A minimum of three samples should be evaluated for each lot. For paper strip BIs, or self-contained BIs with a paper carrier, the carrier should be transferred to an appropriate volume of diluent and pulped into its

component fibers. After pulping, the suspension should be heated at 65-70°C for 15 minutes for *B. subtilis var. niger* spores, or for 15 minutes at 95-100°C for *B.stearothermophilus* or other spore strains used to monitor steam sterilization processes. The heated suspension is then diluted appropriately, plated with soybean casein digest agar or equivalent, and incubated at the manufacturer's recommended temperature for 18-24 hours. The suspension is diluted to yield between 30 and 300 colony-forming units (CFUs) per plate. After incubation, the number of CFUs should be determined and multiplied by the appropriate dilution factor in order to determine the spore population per BI unit.

For spore suspensions and sealed ampule BIs, a sample of the suspension should be aseptically removed and transferred to the appropriate volume of diluent. The diluted sample should then be heat shocked, plated, incubated, and quantitated as described above for paper carrier BIs.

6.5 Resistance performance verification

6.5.1 General

The resistance characteristics of biological indicators to moist heat sterilization are quantified in terms of their D value and the survivor-kill profile. For some types of BIs in certain applications, the determination of the z value may also be necessary, although it is not standard practice for BI manufacturers to determine the z value for each lot of indicators. The resistance of BIs to EO sterilization should be quantified in terms of the D value as well as the survivor-kill performance.

The resistance of the BI to the sterilizing agent should be determined in a BIER/steam vessel (normative reference 2.1) or a BIER/EO gas vessel (normative reference 2.2). Use of any other steam or gas vessels for determining resistance characteristics may yield different results. The procedures used to determine resistance performance characteristics are described in sections 6.5.3, 6.5.4, and 6.5.5. For additional information regarding the determination of resistance performance characteristics see annex B (Pflug 1990).

6.5.2 Acceptance criteria

The manufacturer of BIs shall document the acceptance criteria as well as the test procedures used to determine whether BIs meet these acceptance criteria. Users of BIs should also document their acceptance criteria for BIs and the test procedures used to determine acceptability. If the user accepts the manufacturer's testing criteria, the user may also accept a lot of BIs based upon the Certificate of Performance from the vendor without doing additional testing. A user who does not accept the manufacturer's testing criteria should perform acceptability testing or have this testing performed by a qualified laboratory.

6.5.3 Verification of D value

The D value is defined as the time (in minutes) required under a specific set of conditions to cause a 1-logarithm or 90% reduction in the microbial population (see Figure 1). The D value can be determined by: (1) enumeration analysis; or (2) fraction-negative analysis (see Figure 2).

NOTE — The D value obtained by the two different methods may vary significantly because each evaluates a different area of the response curve. There may be significant differences in the recovery capability of the two procedures; hence, when verifying a D value, it is crucial to mimic the method used by the BI manufacturer as closely as possible. The fraction-negative test is recommended because, in most applications, this is the area of the response curve that is most often of concern. This is also the method used by most BI manufacturers.

The enumeration analysis method is used when knowledge of the upper portion of the response is required. If a shoulder on the enumeration curve is detected, then the D value is calculated from the straight line portion of the curve. To resolve the differences of the shoulder effect, see Pflug 1990.

Self-contained BIs are designed for positive-negative tests using the media provided; therefore, the fraction negative method is the appropriate method for verifying the D value of self-contained BIs.

6.5.3.1 Verification of EO D value using enumeration analysis

D value testing is performed at exposure conditions of 600 ±30mg EO/liter, 54 ± 1°C, and relative humidity of 60 ± 10%. Determination of the D value at other exposure conditions may be necessary depending upon end-use application of the BI. Determination of the D value at the exposure conditions listed above shall be performed according to the following procedure.

a) Randomly select a group of units from each lot of BIs being tested. The size of the selected group may vary depending upon the number of exposure times and number of units exposed per time period, but a minimum of 18 samples will normally be required (i.e., three units per each of six exposure times).

b) Place a minimum of three BI units in an appropriate holder. The holder should permit each BI to be exposed to the EO exposure conditions in the BIER/EO gas vessel.

c) Preheat the BIER/EO gas vessel to 54 ± 1°C.

d) After pressure exhaust of the preheated chamber, and within 15 seconds after opening the chamber, place the sample holder with its BIs into the chamber, seal the door, and initiate the exposure process.

e) Subject the BIs to a 5-minute heat up dwell time at 54 ± l°C.

NOTE — This will allow the equilibration of temperature in the chamber to prevent moisture condensation on surfaces cooled by rapid evacuation.

f) Evacuate the gas vessel to 100 ± 3 mm Hg.

g) Inject sufficient moisture to attain a relative humidity of 60 ± 10%.

h) Subject the BIs to a 30-minute heated humidification dwell time.

i) Inject sufficient preheated EO to attain 600 ± 30 mg EO/liter at 54 ± 1°C in the chamber.

NOTE — During pressurization and evacuation stages in a sterilization cycle, temperatures may vary more than ± 1°C (± 1.8°F) from the preselected temperature value.

j) Begin timing the gas exposure when the gas concentration in the chamber reaches 600 ± 30 mg EO/liter.

k) After the defined exposure period, evacuate the gas vessel to at least 250 ± 10 mm Hg, and release the vacuum with filtered air; repeat this step two or more times.

l) Remove the BI sample holder from the chamber immediately after the door is opened. After removal, BIs should be held in a well ventilated area isolated from any source of desorbing EO.

m) Repeat steps b through l for the subsequent exposure times. For each survivor curve that is to be developed the number of surviving organisms is determined at six to ten exposure times. Exposure times are chosen so that survivor data are developed from an initial concentration of microorganisms to not less than a 3 Log_{10} range.

n) Within 2 hours after removing the BI units from the exposure chamber, aseptically remove the inoculated carrier from its primary package and transfer to a container with a known volume of sterile diluent. Determine the mean number of surviving organisms per EO exposure time as described in steps l and m of section 6.5.3.2.

o) Determine the D value as described in steps n(1) and n(2) of section 6.5.3.4.

6.5.3.2 Verification of moist heat D value using enumeration analysis

D value testing is performed in a BIER/steam vessel at an exposure temperature of 121.1°C (D_{121}). Determination of the D value at other exposure temperatures (D_t) may be necessary depending upon the end-use application of the BI. Determination of the D value at 121.1°C shall be performed according to the following procedure.

NOTE — A shoulder effect may be detected when using the enumeration analysis method at the early exposure time. This can occur either because of the heat-activation characteristics of the spores (Pflug & Holcomb 1983 and Shull 1963) or the heat sink characteristics (Joslyn 1983) of the BI.

a) Randomly select a group of units from each lot of BIs to be tested. The size of the selected group may vary depending upon the number of exposure times and number of units exposed per time period, but will normally require a minimum of 18 samples (i.e., three units per each of six exposure times).

b) Place a minimum of three BI units in an appropriate holder. The holder should permit each BI to be exposed to flowing steam in the BIER/steam vessel.

c) Preheat the BIER/steam vessel to 121.1 ± 0.5°C.

d) Immediately after pressure exhaust of the preheated chamber, and within 15 seconds after opening the chamber, place the sample holder with the BIs into the chamber. Seal the door, and initiate the exposure process.

NOTE — When certain types of self-contained BIs are being evaluated, a vacuum drawn to approximately 1 psia is necessary prior to exposure. Without the prevacuum phase, entrapped air in the unit will affect the observed survival rate (Reich & Whitbourne 1979).

e) Begin timing the exposure when the chamber has attained a temperature of 121.1 ± 0.5°C.

NOTE — The chamber come-up time (time to attain set-point temperature) must not exceed 10 seconds.

f) After the defined exposure time, exhaust the chamber. The chamber must return to ambient pressure within 5 seconds.

g) Within 10 seconds of cycle completion, remove the BI from the chamber.

h) Cool the BI sample to room temperature as soon as possible.

i) Repeat steps b through h for the subsequent exposure times. For each survivor curve that is to be developed, the number of surviving organisms is determined at six to ten heating times. Heating times are chosen so that survivor data are developed from the initial concentration of microorganisms of the sample over a 3 Log_{10} range.

j) After the units have cooled, but within 2 hours, aseptically remove the inoculated carrier from its primary package and transfer it to a container with a known volume of sterile diluent.

k) For paper carrier BIs, or self-contained BIs including a paper carrier, the carrier should be pulped into its component fibers. For other types of carriers, such as aluminum or plastic, consult the manufacturer for the recommended procedure for removing the spores from the carrier material.

l) The spore suspensions obtained from each unit should then be diluted appropriately, plated onto soybean casein digest agar or the equivalent, and incubated at the manufacturer's recommended temperature for 18-24 hours. The suspension should be diluted to yield between 30 and 300 CFUs per plate. After incubation, the number of CFUs should be determined and multiplied by

the appropriate dilution factor in order to determine the surviving spore population per BI unit.

m) Calculate the mean number of surviving organisms for each exposure time. These data can then be plotted graphically using semi-logarithmic graph paper as seen in Figure 2. Similarly the data can be plotted on arithmetic graph paper by calculating the log to the base ten for the number of survivors at each exposure time. From the "straight-line" portion of the graph drawn visually to the points plotted, determine the mean exposure time required to reduce the number of survivors per specimen by 90% (l-log cycle unit).

n) Alternatively, the D value can be calculated using a linear regression analysis of the data. When linear regression analysis is performed, survivor data points within 1-logarithm of the initial population (N_o) are not included in the analysis. The regression analysis is used to fit a straight line model to the log ten survivors vs. heating time. The procedure makes possible a direct estimate of the slope of the survivor curve line. The D value estimate is calculated by taking the negative reciprocal of the slope estimate.

6.5.3.3 Verification of EO D value using fraction-negative analysis

D value testing is performed in a BIER/EO gas vessel at exposure conditions of 600 ± 30 mg EO/liter, at 54 ± 1°C and at a relative humidity of 60 ± 10%. Determination of the D value at other exposure conditions may be necessary depending upon end-use application of the BI. Determination of the D value at the exposure conditions listed above shall be performed according to the following procedure.

a) A group of units is randomly selected from each lot of BIs being tested. The size of the selected group may vary depending upon the number of exposure times and number of units exposed per time period, but will normally require a minimum of 100 samples (i.e., 20 units per each of five exposure times).

NOTE — The number of units run per exposure will depend on both the capacity and the operating characteristics of the BIER vessel being used. It may be necessary to run several exposures at each exposure time in order to test the total number of units required for D value analysis.

b) Place a minimum of 20 BI units (but usually no more than 40) in an appropriate holder. The holder should permit each BI to be exposed to the EO exposure conditions in the BIER/EO gas vessel.

c) Preheat the BIER/EO gas vessel to 54 ± 1°C.

d) Immediately after pressure exhaust of the preheated chamber, and within 15 seconds after opening the chamber, place the sample holder with its BIs into the chamber, seal the door, and initiate the exposure process.

e) Subject the BIs to a 5-minute heat-up dwell time at 54 ± 1°C.

NOTE — This will allow the equilibration of temperature in the chamber to prevent moisture condensation on surfaces cooled by rapid evacuation.

f) Evacuate the gas vessel to 100 ± 3 mm Hg.

g) Inject sufficient moisture to attain a relative humidity of 60 ± 10%.

h) Subject the BIs to a 30-minute heated humidification dwell time.

i) Inject sufficient preheated EO to obtain 600 ± 30 mg EO/liter at 54 ± 1°C, in the chamber.

NOTE — During pressurization and evacuation stages in a sterilization cycle, temperatures may vary more than ± 1°C (± 1.8°F) from the preselected temperature value.

j) Begin timing when the chamber pressure correlates to the gas concentration in the chamber of 600 ± 30 mg EO/liter.

k) After the defined exposure period, evacuate the gas vessel to at least 250 ± 10 mm Hg, and release the vacuum with filtered air; repeat this step two or more times.

l) Remove the BI sample holder from the chamber immediately after the door is opened. After removal, BIs should be held in a well-ventilated area isolated from any source of desorbing EO.

m) Repeat steps b through l for the subsequent exposure times.

For each D value analysis, a minimum of five exposure times should be utilized. Ideally, one exposure time should yield total BI survival, one should yield total BI kill, and two or more should yield partial survival data.

n) Within 2 hours after removing the BI units from the exposure chamber, aseptically transfer each inoculated spore carrier to a test tube containing 5 ml, or an adequate volume, of sterile soybean casein digest broth or the equivalent. For self-contained BIs, activate the unit to allow the spore carrier to be immersed in the growth medium.

o) Incubate the cultured BI units at the manufacturer's recommended temperature.

p) Read and record the results after the manufacturer's recommended incubation time or at scheduled intervals for 7 days.

q) After recording the results, discard the BIs as you would other microbiological waste or according to the manufacturer's instructions.

r) Calculate the D value as described in step n of Section 6.5.3.4.

6.5.3.4 Verification of moist heat D value using fraction-negative analysis

Fractional-negative results are obtained when, from a number of BIs subjected to an exposure cycle, some are determined to be positive for growth and some are determined to be negative for growth. The analysis of fraction-negative data requires two separate data determinations: (1) the initial number of organisms present, and (2) the response, in terms of fractional-negatives, of the BI test units.

Data in the fraction-negative range are analyzed by one of two primary methods: (1) analysis based on the mean time until sterility — Spearman-Karber, and (2) analysis based on the most probable number of survivors — Stumbo, Murphy, and Cochran.

D value testing is performed in a BIER/steam vessel at an exposure temperature of 121.1°C. Determination of the D value at other exposure temperatures (D_t) may be necessary depending upon end-use application of the BI. Determination of D_{121} shall be performed according to the following procedure.

a) Randomly select a group of units from each lot of BIs being tested. The size of the selected group may vary depending upon the number of exposure times and number of units exposed per time period, but a minimum of 100 samples will normally be required (i.e., 20 units per each of five exposure times).

b) Place a minimum of 20 BI units in an appropriate holder. The holder should permit each BI to be exposed to the flowing steam in the BIER/steam vessel.

NOTE — The number of units run per exposure will depend on both the capacity and the operating characteristics of the BIER vessel being used. It may be necessary to run several exposures at each heating time in order to test the total number of units required for D value analysis.

c) Preheat the BIER/steam vessel to 121.1 ± 0.5°C.

d) Immediately after pressure exhaust of the preheated chamber, and within 15 seconds after opening the chamber, place the sample holder with its BIs into the chamber, seal the door, and initiate the exposure process.

NOTE — When certain types of self-contained BIs are being evaluated, a vacuum draw to approximately 1 psia is necessary prior to exposure. Without the prevacuum phase, entrapped air in the unit will affect the observed survival rate.

e) Begin timing the exposure when the chamber has attained a temperature of 121.1 ± 0.5°C.

NOTE — The chamber come-up time (time to attain set-point temperature) must not exceed 10 seconds.

f) After the defined exposure time, shut off the steam flow and open the chamber exhaust line. The chamber must return to ambient pressure within 5 seconds.

g) Within 10 seconds of cycle completion remove the BI from the chamber.

h) Immediately cool the BI sample to room temperature.

i) Repeat steps b through h for the subsequent exposure times. For each D value analysis, a minimum of five exposure times should be utilized. Ideally, one exposure time should yield total BI survival, one should yield total BI kill, and two or more should yield partial survival data.

NOTE — At higher temperatures it may be difficult to obtain more than one partial survival data point.

j) After the units have cooled, but within 4 hours, aseptically transfer each inoculated spore carrier to a test tube containing no less than 5 ml of sterile soybean casein digest broth or equivalent. For self-contained BIs, activate the unit to allow the spore carrier to be immersed in the growth medium.

k) Incubate the cultured BI units at the manufacturer's recommended temperature.

l) Read and record the results at the manufacturer's recommended incubation time or at scheduled intervals for 7 days.

m) After recording the results, discard the BIs as you would other microbiological waste or according to manufacturer's instructions.

n) Calculate the D value as prescribed below or using other appropriate methods.

1) Stumbo, Murphy, and Cochran Procedure (Pflug 1990)

The Stumbo, Murphy, and Cochran method requires the following pieces of data: (1) one or more results in the fraction-negative range, each consisting of the heating time (T), the number of units negative for growth (r), and the number of replicates (n) at each exposure time, and (2) the initial number of microorganisms per replicate unit (N_o). See section 6.4.

$$\text{D value (min)} = \frac{T}{\text{Log } N_o - \text{log } [\ln(n/r)]}$$

Where

T =exposure time

N_o =initial number of organisms per replicate carrier unit

n =total number of replicate units at exposure time T

r =number of units negative for growth at T

2) Spearman-Karber Procedure (Pflug 1990)

The Spearman-Karber procedure can be used if the successive heating times differ by a constant time interval (d), in minutes, and if the same number of replicates (n), is exposed at each interval.

The heating interval (T_1, $T_{2...}T_K$) should be chosen to completely cover the fraction-negative region. There must be an initial heating time (T_1 to show zero sterile replicates or r = 0. The last heating time (T_K), must have all sterile replicates or r = n. The test is valid when there are no negative units (r = 0) at the heating interval proceeding T_1 and all negative replicates (r = n) at the heating time subsequent to T_K, and at least two fractional response time intervals (where r is greater than 0 and less than n) between T_1 and T_K. Calculate the mean time (T) until sterility, or the Spearman-Karber estimate, using the formula.

$$T = T_K - \frac{d}{2} - \frac{d}{n} \times \sum_{i=1}^{K-1} r_i$$

Where

T = mean time until sterility (Spearman-Karber estimate)

T_K = first heating time to show all sterile replicates, $r_k = n_k$

i = heating times

d = time interval, in minutes, between heating times (must be constant — see note)

n_i = number of replicate BIs at each heating time (must be constant — see note)

r_i = number of sterile replicates at each heating time

T_i = longest heating time where none of the BIs are negative, r = 0

T_{K-1} = heating time prior to T_K

$\sum r_i$ = sum of sterile replicate BIs (r) at all heating times between T_1 and T_{K-1}

NOTE — This is a special case of the original Spearman-Karber where d and n are constants. Where d and/or n are not constants, the Stumbo, Murphy, and Cochran formula must be used.

Calculate the D value from the formula:

$$\text{D value (min)} = \frac{T}{\text{Log } N_O + 0.2507}$$

Where

T = mean time until sterility (calculated from the previous formula)

N_o = average spore count per BI determined by total viable count (see section 6.4).

The Spearman-Karber calculation procedure also makes it possible to estimate the variance of T and, in turn, to calculate the standard deviation and determine the confidence interval.

Calculate the variance of T (V_T) from the formula:

$$V_T = \frac{d^2}{n^2(n-1)} \times \sum_{T_1}^{T_{K-1}} r_i (n-r_i)$$

Where

d = time interval, in minutes, between heating times

n = number of replicate units at each heating time

r = number of sterile replicates at each heating time

$\sum_{T_1}^{T_{K-1}} r_i(n-r_i)$ = sum of values derived by multiplying r_i times (n-r) for each heating time between T_1 and T_{k-1}

Calculate the standard deviation of T (SD_T) from the formula:

$$SD_T = \sqrt{V_T}$$

Using the lower and upper confidence limits (CL) for T (confidence limits for T are $T \pm 2SD_T$; p = 0.95), calculate the lower and upper confidence limits for D as

$$\text{D value (lower CL)} = \frac{\text{Lower confidence limit for T}}{\text{Log } N_o + 0.2507}$$

$$\text{D value (upper CL)} = \frac{\text{Upper confidence limit for T}}{\text{Log } N_o + 0.2507}$$

NOTE — For non linear curves, see Pflug 1990.

6.5.4 Procedure to determine the survival-kill response characteristic

In addition to the determination of the D value, each lot/batch of BIs shall be evaluated for their survival-kill response characteristics. The survival performance is the labeled exposure time that results in surviving spores on/in each unit of the sample. The kill performance is the labeled exposure time that results in kill of all spores on/in each unit of the sample. Monitoring the survival-kill response characteristics of a lot of biological monitors provides an additional means of assuring the consistent performance of all units within a given batch.

Survival-kill performance characteristics shall be determined in either a BIER/EO vessel or a BIER/steam vessel. Test procedures for performing these exposures are as outlined in sections 6.5.3.3. and 6.5.3.4.,

respectively.

NOTE — The number of units run per exposure will depend on both the capacity and the operating characteristics of the particular BIER vessel that is used. It may be necessary to run several exposures at both the survival and kill times in order to test the required number of units.

6.5.5 Determination of z value

A z value can be obtained from a thermal resistance curve as shown in Figure 3. There are several alternative methods of determining a microbial z value (Pflug 1990).

One method for determining the z value of a BI is as outlined below.

a) Determine the D value of the BI at two or more temperatures (e.g., 110, 115, 120, 125°C). See section 6.5.3 for D value determination methodologies.

b) The data can be plotted graphically using semilogarithmic graph paper as seen in Figure 3. Similarly the data can be plotted on arithmetic graph paper by calculating the log to the base ten for the D value at each exposure temperature. From the straight-line portion of the graph drawn visually to the points plotted, determine the mean temperature increment required to reduce the D value by 90% (one log cycle unit).

Alternatively, the z value can be calculated using a linear regression analysis of the data. The regression analysis is used to fit a straight line model to the $\log_{10}$ D value vs. heating temperature. The procedure makes possible a direct estimate of the slope of the thermal resistance curve. The z value estimate is calculated by taking the negative reciprocal of the slope estimate.

NOTE — The determination of a z value for some self-contained type BIs may not be appropriate due to the effect of varying heat-sink characteristics at different exposure temperatures (Joslyn 1983).

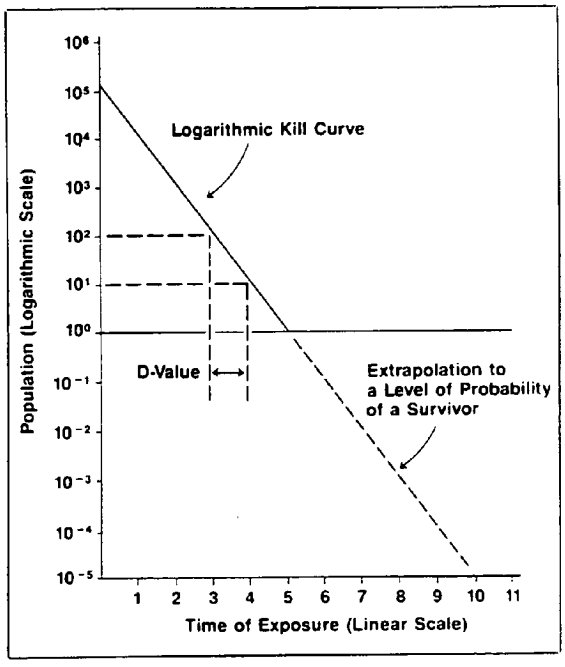

Figure 1 — Kill kinetics of microorganisms

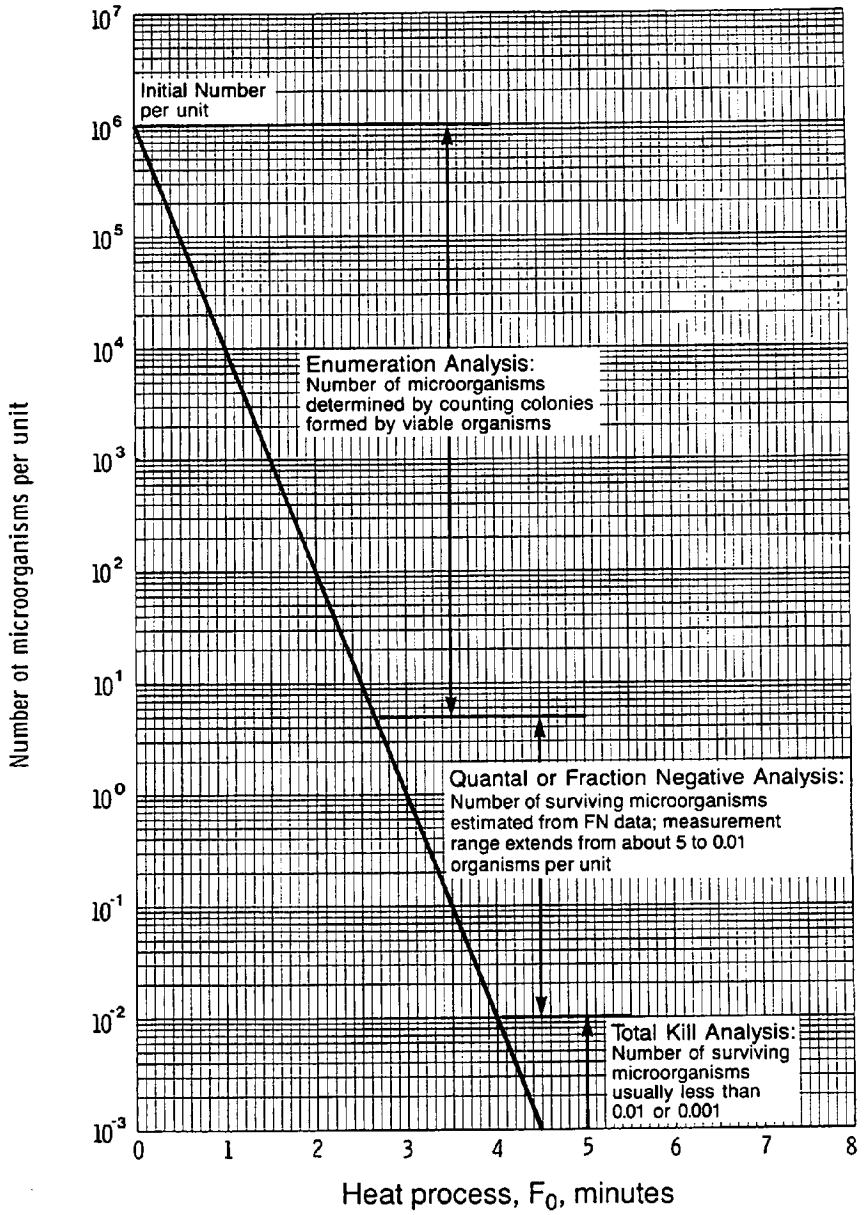

Figure 2 — D value determination

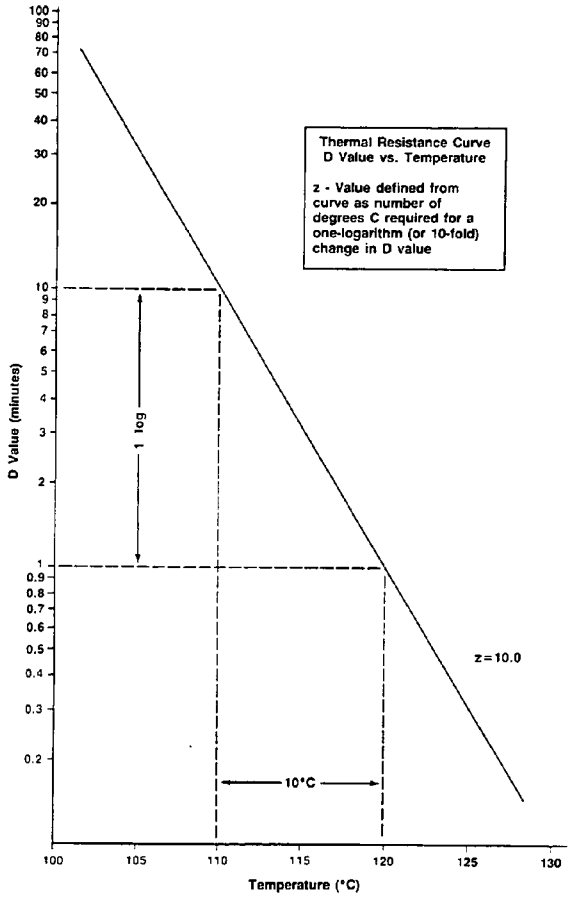

Figure 3 — Z value determination

7 Process qualification — cycle development

This cycle development procedure assumes that the BI has been "calibrated" using the preceding sections of this document. Once this has been accomplished, the BI can be used to measure and compare the relative lethality of sterilization cycles.

Cycle development is the process that determines if a specific sterilization cycle is capable of sterilizing a specified item. It is done in a production chamber with the most challenging load of product. BIs are included in the load and inside the articles to be sterilized. The run is made under production conditions at one-half the exposure time. If no BIs show growth and all physical parameters meet the requirements, the cycle is considered adequate. This approach has the advantage of simplicity and, if all the potential pitfalls are avoided, is sufficient.

Individual components of the product may be evaluated in a research BIER vessel using materials inoculated with a spore suspension, as well as spore strips and manufactured product followed by sterility testing. The tests are repeated in a pilot plant sized sterilizer and finally in a production sterilizer with the most challenging load. The BIER vessel (nominal size 2 ft^3) is used for initial testing to compare the sterilization resistance of the item to that of a test BI in a standard cycle.

A BIER vessel can be used to test the resistance of a BI using a standard cycle. The inactivation kinetics determined in a BIER vessel may not be the same as those seen in a pilot or production vessel. The BIER vessel can only be used to determine the relative resistance of various BIs in its own cycle.

A pilot vessel (nominal size 20 ft^3) capable of reproducing the same cycle as the production vessel may be used to investigate the relative resistance of different products, changed products, and/or determine the master product (most resistant product) in the production cycle. It is also used to develop the cycle and to investigate cycle changes. It can not be employed to evaluate the effects of the load in the production

sterilizer. Cycle effects demonstrated in the pilot vessel should be confirmed in the production vessel.

The production (20 to 5,000 ft^3) vessel must be used with the product in its final form and load configuration to confirm adequate penetration of sterilant and heat throughout the load. It is essential to confirm that significantly different load configurations can be sterilized in the production vessel.

7.1 Product review

The new or changed product must be examined to determine difficult to sterilize sites, which are usually a result of either geometry or materials.

7.1.1 Geometry

The product geometry may cause a particular site to be more resistant to sterilization. The space between the piston and cylinder wall on a syringe is an example of a least-accessible site. A large container of liquid that heats more slowly than a small container or the interior of a tube or a package that is not permeable to gases will also be more resistant to the sterilization process.

Select the site on or in the product that is the most difficult to sterilize as the location for placement of the microbial challenge. It may be necessary to choose multiple locations so that data can be generated to show which sites yield the lowest rate of BI inactivation.

7.1.2 Materials

Material surfaces may contain sites which shelter organisms from the sterilizing environment. There are no general rules that allow one to determine if a material can be sterilized. A hydrophobic material may prevent sterilization by EO because of a lack of moisture at the site. A liquid product may be sporicidal to the inoculated spores. Inactivation of an appropriate spore strip adjacent to the surface in question may be sufficient evidence that sterilizing conditions were present in that location. In some cases prepared BIs are not appropriate and inoculation of the surface with a spore suspension or finished goods sterility testing is necessary.

In cases where the matrix of a material, in addition to the surface, must be sterile, it may be necessary to incorporate spores in the material and then expose the material to a valid sterility test after processing.

7.1.3 Packaging

Product packaging also affects the sterilization process. Large amounts of material (paper, plastic, etc.) may retard the rate at which heat, moisture, and sterilant gas reach the product.

If moisture and EO are required to pass through the packaging material, then porosity should be specified and monitored. Separate testing of the package and/or packaging materials using biological indicators may be necessary.

7.2 BI test unit preparation

After completing the product review and choosing the appropriate sites on or in the product, as well as the type of inoculation to be employed, the BI test unit can be prepared.

Preparation of the BI test unit must be controlled and documented to assure adherence to specifications. Preferably the BI test unit is a replica of the product. If not, additional testing demonstrating a relationship to the product must be conducted. If it is desired to shift from one type of BI to another, then appropriate testing must be conducted.

It should be noted that the biological indicators' certified D value is based upon the biological indicator strips being inside of their primary indicator packaging. When biological indicators are placed within products, the BIs should be utilized without mutilating their primary packaging and/or the BI carrier. Sometimes, however, the biological indicator and its envelope must be folded or twisted in order to be

placed within the product. When the BIs' primary packages are rolled or twisted, the sterilizing conditions are not the same as encountered during manufacturer's certifications. Therefore, in product inoculations with BIs, the D value may not be the same as certified by the manufacturer.

7.2.1 Spore strips

Place the spore strip at the site(s) determined above (7.1.1).

7.2.2 Self-contained BIs (SCBI)

If the size of the SCBI does not preclude its use, it may be utilized in the same manner as the spore strip.

7.2.3 Spore suspension inoculated product

Spore suspensions of the appropriate organisms in specified concentrations may be purchased or prepared in-house.

This method requires operator training and a high skill level. Errors resulting in an increase or decrease of the resistance of the spores on the material are possible. The use of spore suspension inoculated products can produce challenges to the sterilization process that are not typical of either the same spores inoculated on an adventitious carrier or the normal product bioburden (Gillis & Schmidt 1983).

Inoculation of spores on the product may produce a high concentration of spores in a small area and/or location. This can result in clumping, particularly on hydrophobic materials, and/or occlusion on pitted, cracked, or crazed surfaces. Some product site locations, such as O-rings, gaskets, mated surfaces, septum seats and tubing fitments, can also occlude or entrap large numbers of the BI population. Any or all of these factors can delay penetration of the sterilizing conditions to the spore population and result in prolonged survival.

By contrast, typical BI paper carriers provide a large surface area for uniform spore distribution. The mesh-type matrix of the carrier also allows rapid penetration of the sterilizing agent(s) to the spore sites. This allows a consistent measure of the sterilization dose effect against the BI population.

Product bioburden generally results from several sources, including raw materials, manufacturing processes (e.g., ambient air, machinery, human operators, rinse solutions), and packaging materials. This bioburden represents a variation of organism species, states (e.g., vegetative cells or spores), resistance, distribution, and/or concentration on the product. The use of inoculated product will apply a large number of highly resistant organisms to a small area at a specific site. Therefore, the inoculated product approach may produce artificial and misleading results, particularly when used in process validation applications.

If a particular product, because of its geometry or suspected material interaction, requires that BIs be prepared by direct inoculation with a spore suspension, it is highly recommended that expert advice be sought.

7.2.4 Manufactured product

The USP sterility test (normative reference 2.6) is a compendial test, thus the number of samples to be tested will only assure that the compendial requirement for sterility is met. It is not designed, nor should it be used, to determine that a given batch has met a specified SAL.

Similarly, neither BI test units nor product sterility testing can demonstrate that a batch of product has a specified SAL. It only indicates that the cycle has rendered the BIs "sterile" to a 10^{-1} level. To be considered "sterile," a lot must be sterilized to a 10^{-6} SAL for implantables and to a 10^{-3} SAL for topical products. To verify this requires that the BIs be sterile at one-half the exposure time. Therefore, you can only verify that a lot is sterile if you remove the BIs at half exposure time, and this is obviously impractical for routine production (normative reference 2.4).

To assure that a lot is sterile requires validation of the process. Because, with the so-called "overkill" process, the BI is *assumed* to be more resistant than the manufactured product, the sterility of the lot is not assured. To properly "validate the process" requires knowledge of the sterilization resistance of the bioburden of the manufactured product. Cycle times of less than 1/2 exposure time are used for this determination. This validation should be done during cycle development.

This guideline recommends that manufactured product sterility testing in combination with other BI test units be considered a part of all cycle development programs. If periodic bioburden testing confirms that the bioburden has not changed significantly since cycle development, manufactured product sterility testing is optional during routine manufacturing.

When manufactured product is used, it is essential to have a representative bioburden count. It is also important to use more than one lot of material; three lots are recommended. The material should also represent the actual manufacturing product.

7.2.5 BIs for routine monitoring

A BI test unit is a product(s), packaged unit(s), or paper carrier(s) that is inoculated with spores used for cycle development. It is also often the "worst case," "most resistant put-up," or "master product." For this reason it is often inconvenient and costly to use for routine monitoring of production runs.

The desired BI for routine monitoring should be simple, convenient to use and test, and inexpensive. It should also be placed in an easily accessible spot in the load.

The preferred routine BI should be used early in the cycle development program so that a relationship between both the "routine" and the more complex cycle development test unit can be determined. This relationship should demonstrate the relative sterilization resistance of both items.

Exact placement of the BI in the load is required. During qualification runs with production loads, BI test units must be placed in the most difficult to penetrate section of the load (e.g., center of the pallet). During routine manufacturing it is impractical and costly to disassemble pallets for BI placement and then to reassemble them. The relationship between the locations should be demonstrated during cycle development and initial production runs.

7.3 Basic cycle development overkill

The minimum cycle development program consists of three half exposure time runs in a production vessel using temperature sensors distributed in the load of product or simulated product and BI test units as necessary. (See Section 7.1.1.)

7.3.1 BI test units

Prepare a minimum of ten BI test units for each condition found to be necessary in section 7.1 excluding manufactured product samples. More than ten BIs may be necessary to check all conditions.

7.3.2 BI location in load

Distribute the BI test units throughout the load. At least one set must be placed in the center of each loading unit (e.g., pallet, cart, truck), or at that site which is least accessible to the sterilant.

7.3.3 Load

Prepare the load exactly as intended for production runs. Rejected product may be used only if it represents a comparable challenge to the sterilization process. Where EO residuals are concerned, adequate aeration must be provided.

NOTE — If different product loads are to be run, it is necessary to confirm that each can be effectively

sterilized using the same cycle. This can be done by doing half cycle exposures with each load. Alternatively, the "most challenging load" may be used. This concept is analogous to the "master product" concept.

Usually, the "most challenging load" is that load which fills the sterilizer with the heaviest or densest product. Other characteristics, such as number of units, pallet configuration, bioburden, and/or type of material, should also be considered. In some cases, the smallest load may be the most challenging.

It is usually necessary to conduct a study using different load types to determine the "most challenging load."

7.3.4 Temperature sensors

The minimum number of sensors recommended in the AAMI EO and steam guidelines is three sensors for chamber volumes of 100 ft^3 or less and an additional sensor for each additional 100 ft^3 of volume. Use the following to guide exact placement:

a) Place at least one temperature sensor in the expected hot spot(s) of the chamber.

b) Place at least one temperature sensor in the expected cold spot(s) of the chamber.

c) Place additional temperature sensors inside products located in the center of each loading unit (e.g., pallet, cart, truck).

d) If desired, distribute other temperature sensors adjacent to the BI locations listed in 7.3.2.

7.3.5 Exposure times

Make runs at half or less of the intended exposure time for an overkill cycle.

7.3.6 Requirements

If all the BI test units are sterile and the runs have been executed at or near the specified minimum values, then the runs have demonstrated that the cycle is reproducible within the intended limits of the specification. The load and the sterilizer also have met the cycle development and performance qualification requirements. *Any* major changes to the equipment, the product, or the load will require additional qualification testing.

NOTE — It may not be possible to control the sterilizer at a minimum specification value. In such a case, an appropriate additional safety factor must be added to the cycle.

7.4 BI/bioburden cycle development

The combined BI/Bioburden approach to sterilization is based on combining information about a defined microbial challenge (BI) relative to information about the presterilization microbial population (bioburden) of the products or material to be sterilized. This relationship is then employed to select exposure conditions that ensure the inactivation of the bioburden to a predetermined level. This approach assumes that both the BI and the bioburden exhibit exponential inactivation kinetics.

Determination of bioburden levels is based on the recovery of viable populations of microorganisms or their spores from the product at the step in the manufacturing process immediately prior to sterilization. Given the variables associated with any sampling scheme, it is unlikely that the data obtained reflect the true bioburden of the product. Consideration should therefore be given to such factors as the efficiency of the sampling process, the enumeration medium used, incubation temperatures, and heat shock procedures so that factors such as endospore populations can be accurately estimated. The effect of storage conditions and time on the bioburden levels associated with the product should also be considered. Whatever sampling method is selected, it must be validated.

Another concern or potential "pitfall" is the possibility of false positives as a result of inadvertent contamination during sterility testing. To minimize this possibility, the sterility test rooms, procedures, and

personnel must be of high quality, and the exposure time must be minimized to reduce the possibility of inadvertent contamination during testing.

The sterilization resistance of the bioburden and of BIs may be determined using methodologies consistent with those described in Section 6.5.3. Theoretically, this could be done by culturing isolates of the naturally occurring organisms and by inoculating cultures on the product. This method has been criticized because the isolates may change their sterilization resistances when they are cultured. Alternative methods may also be appropriate, but guidelines for this approach are beyond the scope of this document. Any such program, however, must demonstrate a relationship between the naturally occurring organisms and their cultured "progeny."

A comparison is made between the population and resistance of the BI used to monitor the sterilization process and the population and resistance of the bioburden associated with the product. This comparison should demonstrate that inactivation of a predetermined level of BI organisms adequately ensures that the probability of survival of any bioburden organism does not exceed the desired level of sterility assurance for the process (e.g., 10^{-6}) (see figure 4). The challenge species ordinarily used is *Bacillus subtilis* subsp. *niger,* at a population level greater than or equal to the maximum observed product bioburden, but in no case less than 10^3. An example of an initial population might be the average bioburden plus three standard deviations.

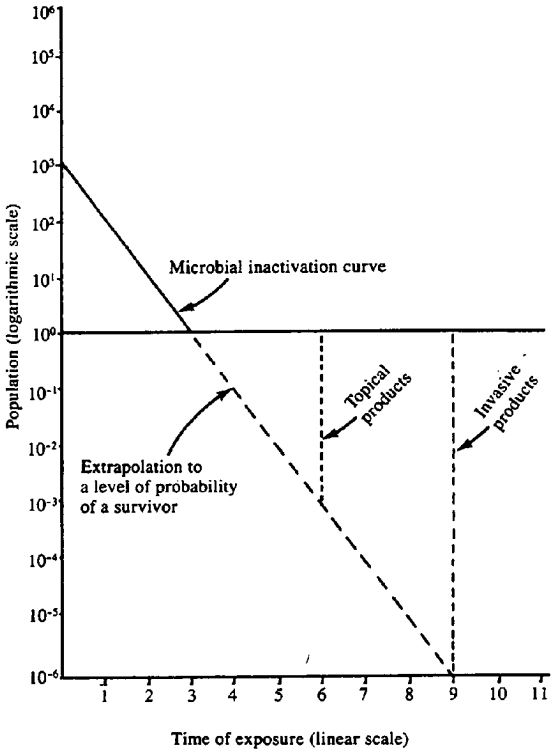

Figure 4 — Combined biological indicator/bioburden method or absolute bioburden method.

Implementation of the combined BI/bioburden technique requires that the user give consideration to many factors including:

a) identification of those microorganisms within the bioburden which present the greatest challenge to the sterilization process

b) the effect of seasonal fluctuations on the bioburden population

c) presterilization conditions that may affect the bioburden population

d) the effect of the sampling method employed (e.g., washing, sonication, blending) on the total bioburden population recovered

e) the effect of incubation conditions (e.g., media, temperature) on the total bioburden population recovered

f) determination of a statistically significant sample size of product for analysis

g) the frequency with which bioburden population and resistance should be monitored

h) validation of the sampling and enumeration methods employed

7.5 Determination of cycle exposure time

The procedure in 7.5.1 below is recommended for BIs, but may also be used for bioburden. Also, see normative references 2.4 and 2.5.

This procedure does not take into account gas charging time or load heating time with moist heat. See section 7.5.2.

7.5.1 The reduced process test method of process design

NOTE — The following section has been adapted and reproduced, with permission, from: Pflug, I. J. *Syllabus for an Introductory Course in the Microbiology and Engineering of Sterilization Processes.* 4th ed. St. Paul, MN: Environmental Sterilization Services, 1980.

The reduced process test method of process design is specifically useful for sterilization procedures that, through some unique attribute of the method or system, can only be evaluated in the field. Ethylene oxide sterilization processes are the ideal example because microbial kill is controlled by the movement of gas and water vapor to the test site as well as the temperature at the test site. Since in ethylene oxide sterilization it is the loading in the autoclave in the plant that determines the length of time required for an adequate kill for a specified temperature and ethylene oxide gas concentration (assuming relative humidity is greater than 30-40%), there is no effective way of duplicating the field situation in the laboratory because it is the physical geometry of the field system situation that determines delivery of the process to the product.

One approach to the use of reduced process testing for sterilization process design purposes is illustrated in figure 5. If the initial microbial load per unit (this will usually be the BI load on a carrier or inoculated onto a product) is known, and the product in its normal loading configuration in the sterilization autoclave is subjected to several different cycles to obtain at least one fraction-negative result, then this process time can be related to a certain number of surviving organisms. (This will be a point in the quantal range.) A line can be extrapolated through these two points (the initial N_o point and the point in the quantal range) to a PNSU of 10^{-6}.

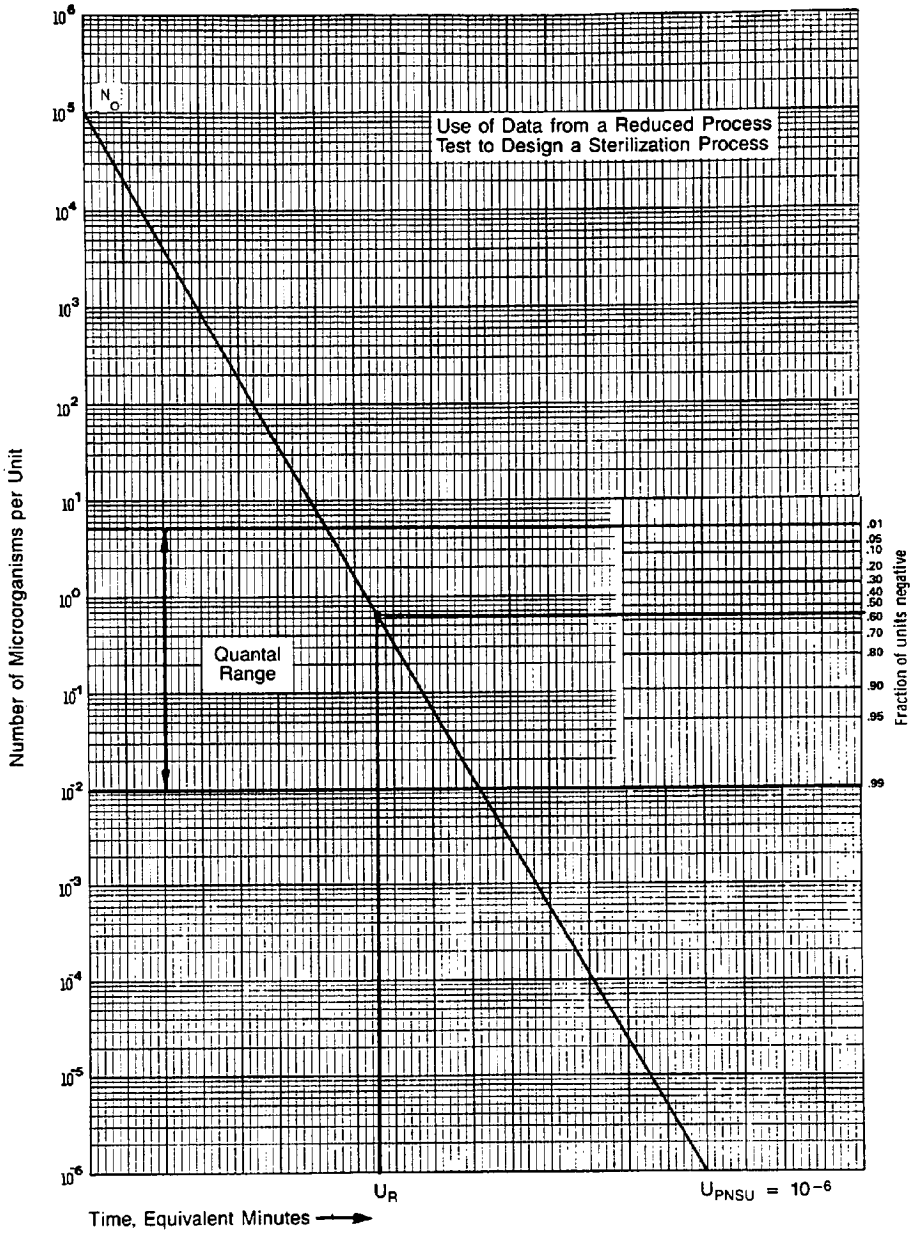

Figure 5 — Use of data from a reduced process test to design a sterilization process

7.5.2 Cycle time reduction value — CTR (Caputo 1980)

As sterilization vessels become larger they are less able to produce "square wave" cycles. That is, a cycle with little or no "come-up" and "come-down" time.

The D value of a BI determined in a BIER vessel designed to have square wave ability will differ from the D value in a production vessel. Steam heat-up and EO "gassing" or "charging" will add lethality to a cycle and make this exhibited D value smaller in a large vessel when compared to a small vessel.

This phenomena may be treated in two ways. One way is to determine the lethality added by the slower "come-up" and "come-down" time. The second way is to use the exposure time determined in the pilot vessel and accept the additional lethality in production as an additional safety factor.

For moist heat cycles, the additional lethality may be calculated using "z" values and "F_0."

For EO cycles there is no method comparable to the steam calculation and the added lethality must be determined experimentally.

Determining the contribution of the come-up and come-down times will allow the shortest exposure time. Ignoring these effects will cause the SAL to be greater in the production vessel than predicted by the pilot vessel.

In either event it is recommended that minimum come-up and come-down times be established in the production vessel specification.

7.6 Lethality correlation

Pilot plant or small research and development vessels are valuable for comparing the relative sterilizability of new and changed products and for evaluating new or changed sterilization cycles. The closer the pilot vessel can mimic the cycle executed in the production vessel, the more useful will be the data.

The principle involved is the use of the BI as a comparative measuring tool to quantify the correlation between cycle lethality in the pilot vessel and cycle lethality in the production vessel. When this relationship is known, it is possible to more effectively plan validation work in the production vessel.

The procedure described below can also be used to quantify the cycle lethality relationship between different production vessels. If the relationship is close, then it may be possible to apply validation data from one vessel to another.

7.6.1 Load

A load in the pilot vessel is optional. If the vessel requires a load for "ballast purposes" (i.e. to stabilize heat-up conditions) then use such a load. If the load can be made to simulate a production load then it may also be helpful. Ultimately, a comparison will be made to the production vessel and it is more important to standardize the load than it is to utilize a particular load. Future runs must be made using an identical load.

7.6.2 Runs at minimum parameters

Make at least three runs in the pilot vessel where all the cycle parameters, except exposure time, are run at or close to the minimums of the expected range allowed for the production vessel. Adjust the exposure times so that one run has BI sterility results that are all or mostly non-sterile, one run results in some sterile BIs and some non-sterile BIs, and one run results in all sterile BIs.

7.6.3 Runs at maximum parameters

Repeat 7.6.2 with all cycle parameters at the maximum of the expected range.

7.6.4 Production vessel runs

Put a full load in the production vessel, distribute the BIs and temperature sensors within the load. Run the cycle at nominal set points. Make at least three runs at different exposure times: one run resulting in all non-sterile BIs, one resulting in all sterile BIs, and one run resulting in a mixture of sterile and non-sterile BIs.

7.6.5 Production vessel correlation

If similar production vessels, running to the same specification, can be shown to have equivalent lethality on the same products and the same loads, validation work can be simplified. For example, rather than making three runs in each vessel for a particular validation, it may be adequate to make two runs in one vessel and only one in the second vessel.

8 Production validation

8.1 Pilot vessel

If a pilot vessel is used during cycle development, validation can be simplified in many cases of new or

changed products. If "master product" is used alongside the new or changed product in fractional exposure time runs, the sterilization resistance of both can be compared. This comparison may be sufficient to eliminate the necessity of having full scale production validation runs. This is especially likely in cases where the products are similar and/or the changes are minor.

8.2 Procedure

In all of the cases below, production validation repeats the essential elements of applicable parts of section 7.

8.3 Production loads

Cycle lethality is affected by the sterilizer load. One should not assume that the same lethality can be obtained in similar sterilizers if the loads are different. The most critical factor is load density followed by load geometry. Usually the densest load is the "worst case" and a successful validation with the densest load will validate similar product loads with equal or less dense product and equal or more "open" geometry. To confirm this, runs may also be made with the lightest load. BIs must be placed throughout the load to assure that sterilization is being achieved in each part of the load.

A BI must also be placed in the interior of the most inaccessible part of the product and load. Do not destroy the value of the test by destroying the product in order to place the BI. For example, if a syringe is cut open to receive a BI, it must be re-sealed so it is as resistant to sterilization as in its original state.

8.4 Process reproducibility

The equipment must reproduce the validated cycle consistently. Equipment unable to maintain control of the process variables should not be used for sterilization processes. The SAL of a sterilization process is based on the ability of the equipment to reproduce the validated cycle, therefore equipment in which the specification limits are often violated cannot be assumed to be reproducing the validated process. Records of nonconformances, corrective action programs and trends must be maintained and reviewed. It is not possible to give an exact requirement for nonconformances, a reasonable goal appears to be 1% to 5% nonconforming runs based on the sophistication and resources of the company.

9 Routine monitoring

9.1 Challenge

To properly use a BI for routine monitoring, it is necessary to know that the BI test unit provides a greater challenge than that observed with the bioburden.

9.2 Frequency

BIs are recommended for use in all production cycles. However, the number of BIs may be reduced from that number used in cycle validation.

NOTE — An exception to the use of BIs to monitor the sterilization cycle has sometimes been allowed when the cycle parameters are thoroughly controlled and monitored. This exception is known as process release or "parametric release," and must be acceptable to the U.S. FDA. Process release is rarely done for EO sterilized products and only occasionally for steam processed products.

9.3 Placement

During validation the BIs should be placed in the areas of the load with the greatest resistance to the sterilization process. Those positions must first be determined by experimentation during the cycle development process. The BIs should be placed strategically in the medical products and packaged, to assure that the challenge to the sterilization is comparable or more resistant than the bioburden on the product.

For routine monitoring, ease of retrieval of the BI must be considered. Locations chosen for routine

monitoring must be correlated with the sites used during validation. The product test units and BIs must be clearly marked with position and run number and must be appropriately placed within the load.

9.4 Retrieval

The BIs must be retrieved and cultured at a time and manner consistent with the process validation. In steam sterilization, the BIs should be cooled to ambient conditions as soon as possible, then removed and placed in growth medium. For EO BIs, the BI units should also be cultured as soon as possible after the load is aerated and the BIs are retrieved.

NOTE — EO exposure to personnel must be avoided. Follow OSHA and the industrial firm's safety recommendations for EO.

9.5 Interpretation

9.5.1 Runs may only be released when both the biological test units and other sterility tests (if used) test sterile and the process parameters meet the minimum validated process requirements, except as provided in 9.2.

9.5.2 Identity of the indicator organism should be confirmed for positive BIs. If the identity is confirmed, the BI is recorded as positive and the result should be interpreted as incomplete sterilization of the product lot. The suspect lot should either be resterilized or destroyed. Frequent positive BIs may indicate an invalid process, a defective BI, or a faulty test system.

When positive cultures are not confirmed to be the indicator organism, conduct further investigation. In general, positive cultures should be confirmed to be a test contaminant. If not, the sterilization process may require revalidation.

10 Periodic revalidation

Revalidation must be done on a periodic basis. Typically, revalidation is performed at least once a year; however, other periods may be used depending on the consistency of the total process. Periodic revalidation can confirm that inadvertent change has not occurred.

Biological indicators can be used to measure the current performance of a previously validated sterilization process. Cycle lethality testing repeated using the same process and load as originally employed can confirm that the sterilization process is delivering the same lethality provided during a previous test.

10.1 Changes requiring revalidation

10.1.1 Product modifications

Product modifications that may increase the product's resistance to sterilization may require repeating all or parts of the original validation.

10.1.2 Load changes

Load configuration changes, including changes due to new or changed products that significantly affect gas and/or steam/heat penetration, must be validated in the production vessel.

10.1.3 Major equipment changes

Changes to the sterilizer that may affect cycle lethality must be evaluated using fractional cycles. New vessels require installation qualification (see normative references 2.4 and 2.5). The rate of BI kill must be compared to previous data. The load should be the same as the load used to generate the previous data.

10.1.4 Cycle changes

Significant changes to the cycle parameters may require new production cycle lethality studies.

10.1.5 Sterilizer vessel equivalence

If equivalence has been demonstrated between production vessels executing the same cycle, it is not necessary to do runs in all the vessels.

Annex A
(informative)
Rationale for the development and provisions of this guideline

A.1 Introduction

This annex discusses the need to develop a guideline providing the essential elements for the use of biological indicators in the development, validation, and routine monitoring of industrial steam and ethylene oxide (EO) sterilization processes. The annex also provides rationale for the specific provisions of the guideline.

A.2 Need for the guideline

There have been many articles published in the literature, as demonstrated by the reference section of this document, which provide guidance for performing sterilization processes using biological indicators for validation. However, none of the referenced documents provide a complete and comprehensive coverage of the use of biological indicators in vessel qualification, validation, and monitoring of a sterilization process, or for the interpretation of the biological indicator results. This document is written to bring all of the components of a validated sterilization process together into one comprehensive treatise for those who are doing sterilization work using biological indicators and for those who must understand the process and interpret the results. Although it will provide useful information to health care facilities, it is primarily designed for the manufacturers of medical products. Because steam and ethylene oxide are the primary sterilization processes used—except for radiation sterilization which is covered in the AAMI recommended practice *Process Control Guidelines for Gamma Radiation Sterilization of Medical Devices*—this document has been written to cover these processes.

A.3 Rationale for the specific provisions of this guideline

A.3.1 Equipment

The section on equipment (section 4) provides the user with a description of commonly used test equipment and the operating parameters of test vessels. This is not meant to be all-inclusive as some companies may require the use of specific parameters with their process. The information in this section can be used to establish an effective sterilization process. This document provides a step-by-step procedure for evaluation of BIs that are to be used for sterilization validation while the BIER vessel documents (normative references 2.1 and 2.2) discuss only the operating parameters.

A.3.2 Selection of biological indicators

The selection of biological indicators is critical to the interpretation of the test results of a process. The resistance of the biological indicator to the sterilization process must be known as well as the initial microbial count in order to properly interpret results. The various methods of sterilization validation are described to provide the user of this document with commonly utilized approaches.

A.3.3 Qualification and validation of biological indicators

A description of the qualification and validation of biological indicators is provided to allow the purchaser to make audits of BI manufacturers and to understand the test results identified on the performance certificate.

Procedures for developing sterilization processes using biological indicators are described, as well as the placement in the sterilization load, the retrieval, and the interpretation of results.

A.3.4 Over- or under-processing

Over- or under-processing may render a product unusable, ineffective, or non-sterile. The contents of this document will provide guidance to medical product manufacturers on the use of biological indicators in the design, development, and monitoring of sterilization processes. This, in turn, will help to assure that products are safe and effective.

Annex B
(informative)
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