Guidance for Industry and FDA Staff

Biological Indicator (BI) Premarket Notification [510(k)] Submissions

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Center for Devices and Radiological Health

U.S. Department Of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Infection Control Devices Branch
Division of Anesthesiology, General Hospital, Infection Control, and Dental Devices
Office of Device Evaluation

Preface

Public Comment

Written comments and suggestions may be submitted at any time for Agency consideration to the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Alternatively, electronic comments may be submitted to http://www.fda.gov/dockets/ecomments. When submitting comments, please refer to Docket No. 2001D-0193. Comments may not be acted upon by the Agency until the document is next revised or updated.

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Contains Nonbinding Recommendations

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Guidance for Industry and FDA Staff

Biological Indicator (BI) Premarket Notification [510(k)] Submissions

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

1. Introduction

FDA regulates biological indicators (BI) intended to monitor sterilizers used in health care facilities as class II medical devices requiring premarket notification (510(k)). 21 CFR 880.2800(a). This guidance document provides information that will help manufacturers prepare 510(k)s for BIs used with conventional sterilization methods. FDA believes that providing this information will promote a consistent and efficient regulatory process.

The effective performance of sterilizers used in health care facilities is important to help prevent nosocomial infections. BIs can provide sterilizer users with information on the effectiveness of sterilizer processes. The use of comprehensive, scientifically sound criteria to evaluate BIs helps ensure the performance of these devices. This guidance document draws upon long-standing scientific principles used to evaluate BIs. It was produced through interaction with industry, government, academia, and health care professionals.

This document supplements other FDA documents regarding the specific content requirements of a 510(k) submission. You should also refer to: 21 CFR 807.87; the guidance, **Format for Traditional and Abbreviated 510(k)s;**[1] and the section of CDRH's Device Advice, **Premarket Notification 510(k)**.[2]

In addition, **The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications**,[3] describes options for submitting either

1 http://www.fda.gov/cdrh/ode/guidance/1567.html.
2 http://www.fda.gov/cdrh/devadvice/314.html.
3 http://www.fda.gov/cdrh/ode/parad510.html.

an Abbreviated 510(k) or a Special 510(k), in lieu of a Traditional 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly once FDA has issued a guidance document addressing that device. Manufacturers considering certain modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to follow the guidance and address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that there is a less burdensome way to address the issues, you should follow the procedures outlined in the **A Suggested Approach to Resolving Least Burdensome Issues**, http://www.fda.gov/cdrh/modact/leastburdensome.html.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

2. Scope

This document provides guidance concerning the content and format of 510(k) submissions for BIs intended to monitor sterilization processes used in health care facilities (product code, **FRC**). These are Class II devices identified under 21 CFR 880.2800(a):

> A biological sterilization process indicator is a device intended for use by a health care provider to accompany products being sterilized through a sterilization procedure and to monitor adequacy of sterilization. The device consists of a known number of microorganisms, of a known resistance to the mode of sterilization, in or on a carrier and enclosed in a protective package. Subsequent growth or failure of the microorganisms to grow under suitable conditions indicates the adequacy of sterilization.

FDA encourages you to contact the Division of Small Manufacturers, International, and Consumer Assistance (DSMICA) or the Division of Anesthesiology, General Hospital, Infection Control, and Dental Devices (DAGID) representatives with any questions you may have before submitting a 510(k) for a BI.

Exclusions

This document does not address the following sterilization process indicators or related products:

- physical/chemical sterilization process indicators (JOJ, LRT, MTC) identified under 21 CFR 880.2800(b)
- BIs intended for use in a manufacturing setting
- BIs intended for use in liquid chemical sterilization (product code **MRB**).

3. Definitions

The following are definitions of terms used throughout the document. Many of the definitions have been standardized by organizations such as the International Organization for Standardization (ISO), the American National Standards Institute (ANSI), and the Association for the Advancement of Medical Instrumentation (AAMI). Complete citations for these standards are in section 12, "**References**."

Biological Indicator (BI): Microbiological test system providing a defined resistance to a specified sterilization process (AAMI, 2006a).

Carrier: Supporting material onto which indicator organisms are deposited (AAMI 2006b).

Chemical Indicator: System that reveals change in one or more predefined process variables based on a chemical or physical change resulting from exposure to a process. (ISO, 2006)

D-value: (decimal reduction value): Time or radiation dose required to achieve inactivation of 90% of a population of the test microorganism under stated exposure conditions (AAMI 2006a).[4]

Death Rate Curve (or Survivor Curve): The graphic representation of inactivation against increasing exposure to stated conditions (AAMI, 2006a).

Inactivation: Loss of the ability of indicator organisms to germinate, outgrow, and/or multiply under specified culture conditions (AAMI, 2006a).

Inoculated Carrier: Specified material onto which a defined number of test organisms have been deposited (AAMI, 2006a).

4 This is the definition for D-value in the standard cited. However, radiation sterilization is not discussed in this guidance document because it is not a sterilization method generally used in health care facilities.

Medical Device: As defined in Federal Food, Drug, and Cosmetic Act (the Act) (21 USC §321(h)):

> [a]n instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is-
>
> > 1. recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them,
> >
> > 2. intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or
> >
> > 3. intended to affect the structure or any function of the body of man or other animals, and
>
> which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended purposes.

Pack, Primary: System that protects the inoculated carrier from damage and contamination without preventing penetration of the sterilizing agent(s) (AAMI, 2006b).

Pack, Secondary: Container system in which BIs are packed for transport and storage (AAMI, 2006b).

Resistometer: A test instrument designed to rapidly produce and precisely control critical parameters associated with a given sterilization process.[5, 6]

Sterile: Free from viable microorganisms (AAMI, 2006a).

Sterility Assurance Level (SAL): Probability of a single viable microorganism occurring on product after sterilization (AAMI, 2006a).

Sterilization: Validated process used to render a product free of all forms of viable microorganisms (AAMI, 2006a).

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5 In addition to routine quality system testing of indicator performance consistency, a resistometer is used to characterize cause and effect relationships associated with a given sterilization process and devices used to evaluate the efficacy of the sterilization process.

6 Resistometers were formerly referred to as Biological Indicator Evaluator Resistometer (BIER) or Chemical Indicator Evaluator Resistometer test systems (AAMI 2002).

Survivor Curve: The graphic representation of inactivation against increasing exposure to stated conditions (AAMI, 2006c).

Survival/Kill Window: Extent of exposure to a sterilization process under defined conditions when there is a transition from all BIs showing growth (survival exposure) to all BIs showing no growth (kill exposure) (AAMI, 2006c).

Test Pack (Process Challenge Device): Item designed to simulate products to be sterilized and to constitute a defined challenge to the sterilization process and used to assess the effective performance of the process (AAMI, 2006a)

Z-value: For a thermal sterilization process, the change in exposure temperature that corresponds to a 10-fold change in D-value (AAMI, 2006b).

4. Device Comparison

FDA recommends that you include a section or table comparing the new device to the legally marketed predicate device. The following table is an example of the type of information that you should provide. (See Section VI below for additional information you should provide in your 510(k)).

Table 1 – Device Comparison

ELEMENT	NEW DEVICE	PREDICATE
Intended use • method of sterilization • process parameters		
Organism • spore • species • strain		
Viable spore population		
Resistance characteristics: • D-value • Z-value (thermal only) • Survival/Kill Window		
Shelf-life		

5. Description and Specifications

You should provide a detailed description of the BI. The description should include the general characteristics of the device design as well as the manufacturing specifications. The bulleted list below is an example of the types of information that you should provide for your device.

- indicator organism, genus, species, and strain
- type of BI, e.g., self contained or paper strip
- intended sterilization process, cycle, and parameters
- resistance characteristics: D-values, survival/kill times, Z-values (thermal only)
- specification for spore population
- shelf-life
- carrier material and primary packaging material
- description of the culture medium (for self contained BIs)
- identification of the media, growth, and culture conditions (for non self contained BIs).

Generally, the following spores are used for the sterilization methods listed below in **Table 2**.

Table 2 – Recommended Spores for Sterilization Process

STERILIZATION PROCESS	SPORE (INDICATOR ORGANISM)
Steam	*Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*)
Dry Heat	*Bacillus atrophaeus* (formerly *Bacillus subtilis var. niger*)
Ethylene Oxide	*Bacillus atrophaeus* (formerly *Bacillus subtilis var. niger*)
Hydrogen Peroxide	*Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*)

For BIs used in sterilization methods other than those listed, you should demonstrate that the BI used for validation is the most resistant to the process. Alternatively, you should reference published scientific studies indicating that the subject BI is the most resistant to the process. If you use an organism for a sterilization process that is not specified in Table 2, you should

provide test data to demonstrate that the subject microorganism is at least as or more resistant than that listed in Table 2.

6. FDA-Recognized Standards

If any part of the device design or testing relies on a recognized standard, we recommend that you include either:

- a statement that testing will be conducted and meet specified acceptance criteria before the device is marketed or
- a declaration of conformity to the standard.[7]

Because a declaration of conformity is based on results from testing, we believe you cannot properly submit a declaration of conformity until you have completed the testing the standard describes. For more information, please refer to section 514(c)(1)(B) of the Act and the FDA guidance, **Use of Standards in Substantial Equivalence Determinations; Final Guidance for Industry and FDA**.[8]

Recognized standards that apply to BIs are discussed in the following sections.

7. Performance Characteristics

FDA recommends that you provide the following information in your 510(k).

- clear statement of the study objective(s)
- description of the equipment and media used to evaluate the BI, including the results of the growth promotion studies
- enumeration of viable spore populations
- resistance characteristics of the final finished product, as appropriate to the sterilization method (e.g., D-value, Z-value, survival/kill window)
- sterilization methods, cycle parameters, and exposure times
- determination of growth inhibition of carrier and primary packaging
- assessment of sterilant neutralization effectiveness, if neutralization is required
- positive/negative controls
- summary of study results
- conclusions reached based upon study results.

7 See Required Elements for a Declaration of Conformity to a Recognized Standard (Screening Checklist for All Premarket Notification [510(k)] Submissions), http://www.fda.gov/cdrh/ode/reqrecstand.html.

8 http://www.fda.gov/cdrh/ode/guidance/1131.html.

FDA recommends that performance studies include statistically valid sample sizes and are conducted on at least 3 different spore lots, prepared from different spore crops. You should conduct the performance studies discussed below.

A. Viable Spore Population Assay

You should provide assay results to demonstrate that the product meets its specification for the spore population. For examples of assay methods, refer to the applicable United States Pharmacopeia (USP) standards and FDA recognized standards (see **12. References**). See also **Table 3 Recommended Minimum Populations and Resistance Characteristics**.

B. Resistance Characteristics Study

You should provide the resistance characteristics of the BI in the specified sterilization process and cycle, using a resistometer. We recommend that you perform all testing on only final finished products. We also recommend that the resistometer used for the characterization of the BI conform with the current ANSI/AAMI standard for resistometers. If no resistometer exists for the sterilization process being evaluated, we recommend using actual production sterilizers with the critical cycle parameters controlled to the extent possible during testing. You should document that testing parameters were adequately controlled and explain how this was achieved. If you conduct resistance characteristics studies using sterilizers that do not routinely monitor all critical parameters, we recommend that you add external monitors to document evidence of adequate control.

D-value

For test methods for determining BI resistance characteristics, please refer to ANSI/AAMI ST 59 or equivalent method.

For non self-contained BIs, you should plot the D-values for a representative range (e.g., 115°C, 120°C, 125°C, 130°C) to determine the D-value for different thermal exposure temperatures. If the curve demonstrates linearity, the BI is suitable for the full range of cycle temperatures.

For self-contained BIs, you should calculate the D-value at each proposed temperature.

Z-value

You should provide Z-values for thermal processes. FDA generally recommends that BIs have a minimum Z-value of 10°C.

To determine the Z-value, you should construct a thermal resistance curve. The thermal resistance curve is a semi-logarithmic graph of D-value vs. temperature. The Z-value can be obtained from the slope of the curve by calculating the number of degrees of temperature for a 1-logarithm change in D-value. The greater the number of

temperatures studied, the more accurate the resulting Z-value. FDA recommends that studies use at least three different temperatures (Pflug, 1990).

Survival/Kill Window

You should conduct a study to verify the calculated survival/kill window. USP provides an acceptable test method. The minimum expected survival time and maximum expected kill time can be calculated from the following equations (USP):

Minimum Expected Survival Time

$$\text{Survival Time} = \text{Not Less Than } \text{D - value} \times (\log_{10} \text{ viable spore population} - 2)$$

Maximum Expected Kill Time

$$\text{Kill Time} = \text{Not More Than } \text{D - value} \times (\log_{10} \text{ viable spore population} + 4)$$

Table 3 below lists examples of the resistance characteristics of 510(k) cleared BIs with minimum populations, D-values, and the survival times.

Table 3 - Recommended Minimum Populations and Resistance Characteristics

STERILZATION CYCLE	VIABLE SPORE POPULATION	D-VALUE	Z-VALUE	SURVIVAL TIME
Steam 121°C	10^5	1.5 min	10°C	5 min
Steam 132°C	10^5	10 s	10°C	1 min
Steam 134°C or 135°C	10^5	8 s	10°C	40 s
Ethylene Oxide 600 mg/L, 60% RH, 54°C	10^6	3 min	**A**	15 min
Dry heat 160°C	10^6	3 min	**A**	12 min

A Not applicable.

C. Carrier and Primary Packaging Materials Evaluation

You should evaluate the effects of carrier and packaging materials on the resistance characteristics of the BI. Please refer to ANSI/AAMI/ISO 11138-1 Annex B for examples of methods that may be used.

D. Holding Time Assessment

Holding time is the length of time that the exposed BI is held before incubation. You should evaluate the effect of the labeled holding time on the resistance characteristics and spore recovery.

E. Recovery Protocols

You should identify the recovery media and evaluate all recovery protocols to validate the incubation time. Generally, FDA recommends 7 days as the conventional incubation time for BIs used to monitor the traditional sterilization processes listed in **Table 2**. To validate incubation times of less than 7 days, see **Examples of Validation of Biological Indicator Incubation Time** in **Attachment II**.

For self contained BIs, you should also evaluate the effect of the sterilization process on the recovery medium.

F. Mail-In Protocols

Some manufacturers market BIs as part of a monitoring service; the user mails the exposed BI to the manufacturer (or laboratory) to incubate and read. The handling required by the monitoring service may impact adversely upon the BI resistance. Therefore, labeling for BIs marketed as part of a monitoring service should detail the handling protocol. The handling protocol should be validated.

8. Shelf Life

You should demonstrate that the specifications (e.g., D-value, spore population) are maintained throughout the labeled shelf life of the device. For all studies, FDA recommends that you use at least 3 lots from 3 different spore crops of BI and a statistically significant sample size. You should use storage conditions described in the device labeling.

You should provide the following information about shelf life:

- labeled shelf life
- dates and length of time for each testing interval, e.g., 0, 6 months, 1 year, 2 years
- resistometer vessel results for each testing interval demonstrating that the established endpoints are maintained.

BIs may be unstable at elevated temperatures; therefore, FDA believes accelerated aging studies may be inappropriate.

In lieu of complete real time shelf life data, the FDA will consider preliminary real time shelf life data (e.g., 6 months) along with a detailed protocol and sampling plan for an ongoing real time

(e.g., 2 years) study that you intend to continue after your device is cleared. These study protocols should include the shelf life information listed above. You should document the results in your design history file as a part of the Quality System Regulations (21 CFR 820.30).

9. Incubators

BI labeling may recommend a suitable incubator. Alternatively, labeling may recommend incubation conditions and allow users to select an appropriate incubator. FDA considers incubators without specific claims to be general purpose laboratory equipment regulated under 21 CFR 862.2050 as Class I exempt. A 510(k) may be required for incubators that are dedicated for a specific BI or make specific performance claims that go beyond incubation.

However, incubator performance is an important aspect in BI validation. Some (typically self-contained) BIs are marketed as part of a system that includes an incubator. A 510(k) for this kind of BI should include a certification that any incubator sold as part of a system maintains the operating temperature specifications under all potential loading conditions over the recommended incubation time.

10. Test packs

BIs may be used in test packs to simulate products being sterilized.[9] Test packs are intended to simulate products and constitute a defined challenge to the sterilization process that is equal to or greater than the most difficult item routinely processed. For BIs indicated for use in specific test packs, you should demonstrate that the performance of the BI in that test pack is equivalent to the performance of the AAMI reference BI in the same test pack in their respective sterilization processes. You should also demonstrate that the BI test pack provides a greater challenge to the process than the BI itself.

11. Labeling

The premarket notification must include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). The following suggestions are aimed at assisting you in preparing labeling that satisfies the requirements of 21 CFR Part 801.[10]

A. Intended Use

We recommend that the intended use describe:

- method of sterilization

9 These test packs are also called process challenge devices (PCDs).

10 Although final labeling is not required for 510(k) clearance, final labeling must comply with the requirements of 21 CFR Part 801 before a medical device is introduced into interstate commerce. Labeling recommendations in this guidance are consistent with the requirements of part 801.

- sterilization cycle
- exposure time
- temperature
- sterilant concentration
- any other information specific to the intended use of your device.

B. Description

We recommend that the labeling provide a description of the device. The description should include:

- genus, species, and strain
- lot/batch number
- type of BI (paper strip, self-contained, size of paper carrier)
- D- value and method used to determine it (see also **D. Precautions** below)
- Z-value (thermal processes only)
- survival/kill time under the conditions specified
- total viable spore count, including whether the count had been determined after preliminary heat treatment
- expiration information.

C. Instructions for Use

We recommend that your instructions for use include:

- sterilization method, cycle, and exposure time
- instructions for interpreting results
- instructions for disposal, e.g., "sterilize by steam 121°C for not less than 30 minutes"
- instructions for spore recovery, e.g., incubation time, culture conditions, type of media.

For self contained BIs, we also recommend that the labeling state whether the supplied bacteriological medium will meet requirements for growth promoting ability.

D. Precautions

The labeling should advise users that the manufacturer's D-value cannot be duplicated in the healthcare facility, for example:

The D-value is reproducible only under the exact test laboratory resistometer conditions under which it was determined. The user would not necessarily obtain the same result.

12. References

AAMI 2006a. Association for the Advancement of Medical Instrumentation (AAMI). Sterilization of health care products-Vocabulary. ANSI/AAMI/ISO TIR11139:2006.

AAMI. 2006b. Association for the Advancement of Medical Instrumentation (AAMI). Sterilization of health care products-Biological indicators-Part 1: General requirement. ANSI/AAMI/ISO ST11138-1:2006.

AAMI.2006c. Association for the Advancement of Medical Instrumentation (AAMI). Sterilization of health care products- Biological indicators- Part 4: Biological indicators for dry heat sterilization processes. ANSI/AAMI/ISO ST11138-4:2006.

ISO. 2006. International Organization for Standardization (ISO). Sterilization of health care products Biological and Chemical Indicators Test Equipment. ISO 18472:2006.

Pflug, I. J. 1990. Microbiology and engineering of sterilization processes. Environmental Sterilization Laboratory, 100 Union Street, Minneapolis, MN 55455.

United States Pharmacopeia (USP) & National Formulary. USP 30 (2007) or current edition of the following:

USP Biological Indicator for Dry-Heat Sterilization, Paper Carrier

USP Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier

USP Biological Indicator for Steam Sterilization, Paper Carrier

USP Biological Indicator for Steam Sterilization, Self Contained

Attachment I. Summary of FDA Recommendations

Below is a summary of the recommendations provided in this guidance document. We recommend that you also refer to **Format for Traditional and Abbreviated 510(k)s**.[11]

Device Comparison
Description And Specifications
Genus, species, and strain
Design, e.g., paper strip, self contained
Spore population and concentration
Carrier material
Packaging, primary and secondary
Culture medium
Resistance characteristics
Voluntary Consensus Standards BI meets or will meet
Labeling
Description of design, e.g., paper strip, self contained
Identification of the genus, species, and strain
Conditions for use, including sterilization process parameters (sterilizing agent, concentration, temperature) and (for steam) gravity versus dynamic air removal (pre-vacuum), etc.
Heat shock spore population and concentration
D-value and method used for D-value determination. Z-value, if appropriate. Survival/kill times or any other applicable resistance characteristics
Shelf-life and manufacture or expiration date
Storage conditions with temperature and humidity ranges
Instructions for interpreting results and spore recovery
Instructions for disposal, method, any neutralizers needed, or hazardous wastes associated with product use
The reproducibility of the D-value
Whether bacteriological medium is adequate to promote growth (self contained designs only)
Performance Characteristics
Viable spore population assays
D-value
Z-value
Survival/Kill Time
Carrier and primary packaging materials evaluation
Holding time assessment
Recovery Protocol, medium and incubation time
Effect of sterilization process on recovery medium (self contained BIs only)
Validation of accessories (e.g., incubator, test pack) if any
Validation of mail-in protocols, if any

[11] http://www.fda.gov/cdrh/ode/guidance/1567.html.

Attachment II. Examples of Validation of Biological Indicator Incubation Time

These recommendations are appropriate for all biological indicators, self contained or on a strip, which are intended to accompany products being sterilized through a sterilization procedure and to monitor adequacy of sterilization.

The incubation period for BIs may be reduced from the standard seven or more days, provided that the validation studies demonstrate that the revised numbers of days of incubation are sufficient according to appropriate methodology.

Methodology

FDA recommends that manufacturers use the following methodology to reduce the incubation time for BIs from the standard seven or more days.

- Identify the partial cycle in which 30 -80% of the BIs survive. A partial cycle is one in which all sterilization parameters, except the time parameter, are met.
- Obtain a minimum of 100 BIs from each of 3 different lots (for a minimal sample size of 300 BIs).
- Expose the BIs to 3 partial sterilization cycles using 100 indicators from each lot per each partial cycle. Only the BIs from one lot should be used in each partial cycle. Lots should not be mixed.
- It is preferable to run BIs in a device load. However, because of the inherent difficulties of achieving a partial cycle kill under such circumstances, partial cycles can be run without device loads.

  NOTE: During all sterilization validation studies, the effects of the sterilant in combination with the device material on the indicator organism should be determined. If the materials are judged to have a significant effect on organism destruction, the indicators should be exposed to the sterilant in conjunction with the devices during the partial cycle studies.
- Place the BIs in the growth media no more than 8 hours after removal from the sterilization chamber or removal from the sterilized load of devices. Incubate all of the BIs for a minimum of 7 days.
- Prepare a growth chart to record the number of positive BIs on either a daily basis or for the particular time interval of interest.
- Using the number of BIs that test positive on day 7 as the base of 100% grow out (denominator data), determine from the growth chart the number of BIs that have more than 97% of the base number of BIs (numerator) that test positive in each partial cycle for the proposed incubation time to be acceptable.
- The greatest number of days of incubation time to obtain more than 97% positive BIs (based on the 7 day incubation time) in any one of the partial cycles is the

minimum incubation time for the BI. For this method, averaging the three (or more) partial cycle incubation times is not recommended (see examples below).

- If there are fewer than 30% survivors or more than 80% survivors in any one run, this particular run should not be used and should be repeated to achieve the desired number of survivors (see examples below).

Example #1: The following Table provides examples of the number of positive BIs needed to achieve 97% growth.

Table A – Examples of 97% Grow Out of the Biological Indicator

Numerator Data [1]	30	31	32	33	33	34	35	36	37	38	
Denominator Data[2]	30	31	32	33	34	35	36	37	38	39	
Percent Growth	100%	100%	100%	100%	97%	97%	97%	97%	97%	97%	
Numerator Data	39	40	41	42	43	44	45	46	47	48	
Denominator Data	40	41	42	43	44	45	46	47	48	49	
Percent Growth	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	
Numerator Data	49	50	51	52	53	54	55	56	57	58	
Denominator Data	50	51	52	53	54	55	56	57	58	59	
Percent Growth	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	
Numerator Data	59	60	61	62	63	64	65	65	66	67	
Denominator Data	60	61	62	63	64	65	66	67	68	69	
Percent Growth	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	
Numerator Data	68	69	70	71	72	73	74	75	76	77	78
Denominator Data	70	71	72	73	74	75	76	77	78	79	80
Percent Growth	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%

[1] The numerator is the number of positive BIs that is greater than 97% of the denominator. If the numerator is equal to or greater than the one listed for the corresponding denominator (based on the total number of positive BIs on day 7), the length of the incubation time when this occurs is acceptable.

[2] The denominator is the total number of positive BIs on day 7 of incubation.

Example #2 - A manufacturer would like to reduce its biological indicator incubation time to 3 days. Using the methodology described above, the following data was generated.

Table B – This Manufacturer's Test Data

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Partial cycle #1							
Numerator	0	56	57	57	58	59	59
Denominator	59	59	59	59	59	59	59
Percent Growth	-	94.9%	96.6%	96.6%	98.3%	100%	100%
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Partial cycle #2							
Numerator	1	34	35	35	35	35	35
Denominator	35	35	35	35	35	35	35
Percent Growth	2.9%	97.1%	100%	100%	100%	100%	100%
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Partial Cycle #3							
Numerator	0	79	81	Test invalid because percent positive biological indicators is outside the 30-80% growth.			
Denominator							
Percent Growth							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Partial Cycle #4							
Numerator	0	47	48	49	49	49	49
Denominator	49	49	49	49	49	49	49
Percent Growth	0	95.9%	98.0%	100%	100%	100%	100%

The 3-day reduced incubation time is not validated because, of the three valid partial cycles, not all achieved 97% growth in 3 or fewer days. However, based upon the criteria listed in bullet 7 of the test methodology, the data validate a 5-day reduced incubation time. The 5-day incubation time in this example is the greatest number of days, from all the valid partial cycles, needed to grow out more than 97% of the denominator BIs.