

To Kill or — A biological indicator story Not to Kill

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Managing Infection Control and 3M Health Care will be working collaboratively to provide continuing education courses in monthly editions of *Managing Infection Control*.

Objectives

1. Explain the historical evolution of the role of bacterial spores in monitoring the effectiveness of a sterilization process.
2. Develop an understanding of the minimal performance criteria a biological indicator (BI) must meet and the important concepts of sterilization such as survival/kill, D-value, sterility assurance level (SAL) and spore count.
3. Discuss sterilization process failures detected by BIs.
4. Describe the Association of periOperative Room Nurses (AORN) and Association for the Advancement of Medical Instrumentation (AAMI) recommended practices on the role of BIs in monitoring the effectiveness of the sterilization process.

Questions

True or False. Circle the correct answer.

1. Biological indicators were used to test the effectiveness of a sterilization process as early as 1876.
A. True B. False
2. Biological indicators are accepted universally as a gold standard by several reputed standards organizations for measuring the efficacy of a sterilization process.
A. True B. False
3. Rapid Readout Self-Contained biological indicators are the newest BI technology that directly measure the lethality of a sterilization process.
A. True B. False
4. Waiting for biological indicator results is important because they are the only monitoring tool that has been documented to consistently detect superheated steam, air/steam mixtures and entrapped air.
A. True B. False
5. An SAL of 10^{-6} indicates that a probability of finding a surviving organism is one in a million.
A. True B. False
6. Sterilization process failures are a result of inadequate air removal, poor steam quality, and/or inadequate time at temperature.
A. True B. False

7. The minimum spore population required by the FDA for a steam biological indicator is 1×10^5 (100,000) spores.
A. True B. False
8. Both AORN and AAMI state that a biological indicator should be used weekly, preferably daily, for routine sterilizer efficacy monitoring and for monitoring every load containing an implantable device which should be quarantined until the BI results are available.
A. True B. False
9. AORN and AAMI require that biological indicators also be used for steam sterilizer qualification testing (after installation, relocation, malfunctions, major repairs and sterilization process failures) and for product testing.
A. True B. False
10. AORN and AAMI state that if a biological indicator is positive you should recall all items processed since the last negative BI.
A. True B. False

Introduction

Budgetary and inventory management constraints within the healthcare industry, combined with the ever increasing demands to optimize efficiency in sterilization of surgical instruments, have resulted in a need for faster turn around of instrumentation in the surgical setting. There is an ongoing search to identify the best and most useful monitoring tools that will enable the user to make informed decisions on the effectiveness of the sterilization process. It is well understood that each of the currently available monitors has a role to play and one does not replace the other. However, because of this need for speed, the role of biological indicators (BI) has recently been questioned. This inservice will discuss:

- ▶ Why Spores?—A Historical Perspective;
- ▶ Evolution of Biological Indicators;
- ▶ Is it Worth the Wait for a Biological Indicator;
- ▶ Important Sterilization Concepts;
- ▶ Steam Sterilization Process Failures;
- ▶ Biological Indicator Design;
- ▶ Usage of Biological Indicators.

At the end of this inservice you will be able to discuss why BIs became and continue to be the “**Gold Standard**”, primarily because of their ability to provide a direct measure of the lethality of a sterilization process.

Why Spores?—A Historical Perspective

The concept of sterilization was born during the era of Lazzaro Spallanzini (1729-1799) who demonstrated that sealed flasks boiled for a few minutes have living “animalcules”, but those boiled for an hour did not.¹ Subsequently, John Tyndall demonstrated that bacteria have a heat stable and heat labile form based on his observations of the time it took for effective sterilization by boiling. It was Frederick Cohn in 1877 that described spores of *Bacillus subtilis* and demonstrated their heat resistant properties.

During the same period of 1876-1880, Charles Chamberland developed the first pressure steam sterilizer, or autoclave, with which it was possible to achieve temperatures of 120°C. Robert Koch was instrumental in identifying moist heat to be superior to dry heat because of its greater penetrating power. His ingenious experiments involving the exposure of a roll of flannel contaminated with spores to dry heat at 140-150°C for 4 hours demonstrated the survival and free germination of spores, while complete destruction occurred when the contaminated flannel was exposed to moist heat at 120°C for 30 minutes.² **These experiments can be considered to be the first evidence of the use of bacterial spores as BIs, as we know them today** (see Figure 1).

Figure 1. Spores of *Geobacillus stearothermophilus* used in biological indicators for Monitoring Steam Sterilization Processes



Further advances in steam sterilization came from a number of other sources. Sterilizing surgical dressings was introduced by Schimmelbusch in 1885. The importance of the quality of steam and the negative impact of air on the bactericidal effect of steam was studied by Von Esmarch and Max Rubner. Experiments by George Sternberg (1878) on the death kinetics of non-spore forming pathogenic bacteria (death after 10 min. at 62°C to 70°C) vs. spore formers (death after 5 min. at 120°C) provided early evidence that studying the destruction of spores provided a safety margin to the sterilization process.

Table 1. Organizations recommending the routine use and frequency of monitoring sterilization effectiveness with biological indicators

ORGANIZATION	RECOMMENDATION
Centers for Disease Control and Prevention (CDC) ³	"Monitor sterilizers at least weekly by using a biological indicator with a matching positive control (i.e., BI and control from same lot number)."
American Dental Association (ADA) ⁴	"Biological monitors should be used routinely to verify the adequacy of sterilization cycles. Weekly verification should be adequate for most dental practices."
Centers for Disease Control and Prevention (CDC) ⁵	"Proper functioning of sterilization cycles should be verified by the periodic use (at least weekly) of biologic indicators (i.e., spore tests)."
Centers for Disease Control and Prevention (CDC) ⁶	"The adequacy of sterilization cycles should be verified by the periodic use of spore-testing devices (e.g., weekly for most dental practices)."
Centers for Disease Control and Prevention (CDC) ⁷	"All sterilizers should be monitored at least once a week with commercial preparations of spores intended specifically for the type of sterilizer."
Centers for Disease Control and Prevention (CDC) ⁸	"Use biological indicators to monitor the effectiveness of sterilizers at least weekly with a FDA-cleared commercial preparation of spores intended specifically for the type and cycle parameters of the sterilizer."
Centers for Disease Control and Prevention (CDC) ⁹	"Inadequate sterilization of surgical instruments has resulted in SSI outbreaks. The importance of routinely monitoring the quality of sterilization procedures has been established. Microbial monitoring of steam autoclave performance is necessary and can be accomplished by use of a biological indicator."
Association for the Advancement of Medical Instrumentation (AAMI) ¹⁰	"Biological indicator test packs also should be used routinely in sterilization loads at least weekly, but preferably every day the sterilizer is in use." "Additionally, BIs within PCDs should be used to monitor every load containing implants. Biological indicators within a PCD may be used as part of the criteria for release of nonimplant loads."
Association of periOperative Nurses (AORN) ¹¹	"In an emergency, when flash sterilization of an implant is unavoidable, a rapid-action BI with a Class 5 chemical integrating indicator (or enzyme only indicator) should be run with the load." " Steam sterilizers: <i>Geobacillus stearothermophilus</i> biological indicators should be used for routine sterilizer efficacy monitoring ... Routine sterilizer efficacy monitoring should be done weekly, preferably daily, as follows: <ul style="list-style-type: none"> ▶ Each load containing an implantable device should be monitored with a BI and quarantined until the results of the BI testing are available, and ..."

It is evident from the historical use of bacterial spores that BIs for monitoring sterilization efficacy have roots that are a few centuries old. Furthermore, the role of spores as the direct measure of lethality continues into the 21st century. **Having thus gained the status of a tried and trusted measurement of spore lethality, BIs are now accepted universally as a "Gold Standard" by several reputed standards organizations** (see Table 1).

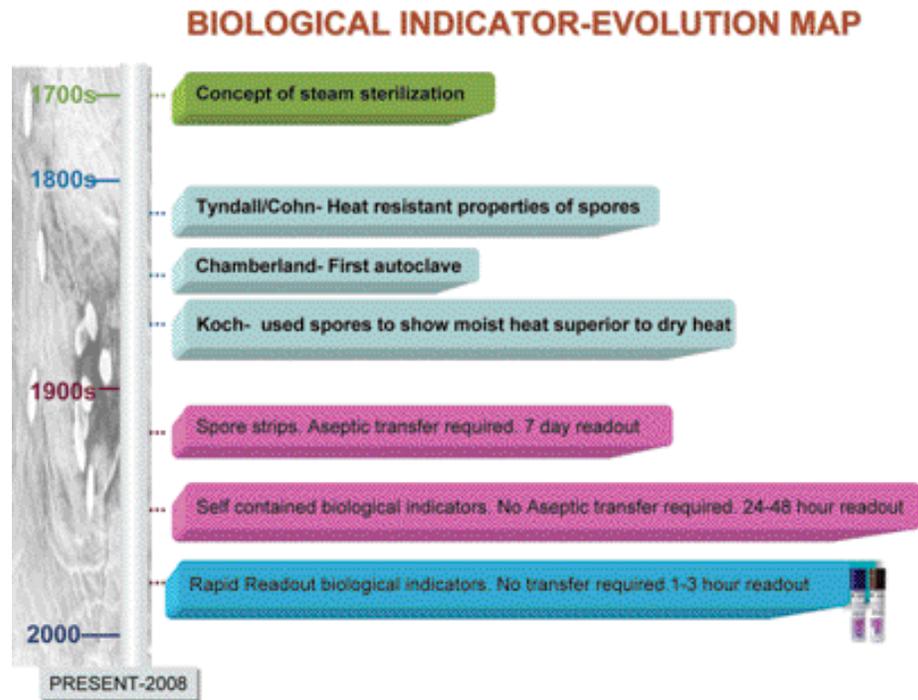
"No professional organization (e.g., AORN) has recommended the use of Class 6 emulating indicators as a substitute for biological indicators and there are no data that demonstrate that it mimics a BI at suboptimal sterilization times."

— Dr. William Rutala

Evolution of Biological Indicators

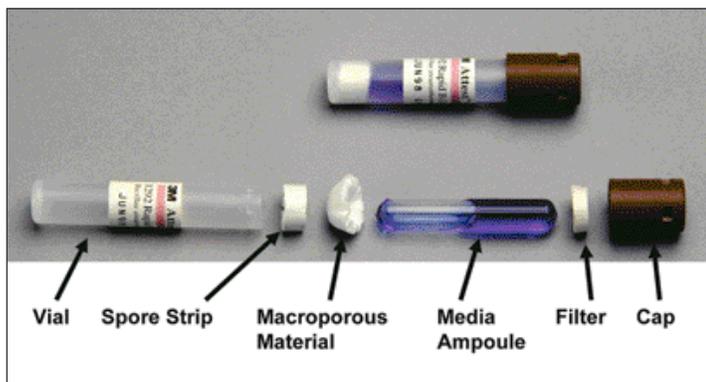
The evolution of BI technology is diagrammatically depicted in Figure 2. Early BIs, primarily spore strips with a known population of spores, had a readout time of 7 days. This meant that information about whether the sterilization process was successful or not was not available to the end user within an actionable time frame. Use of spore strips required the operator to be well trained in aseptic technique. In several instances, incidental contamination during the transfer of a spore strip into media would result in turbidity of the growth media thereby generating a false positive indication. This problem was alleviated by the introduction of self-contained biological indicators (SCBIs) in approximately 1976 which had the spore strip and nutrient media all within a single container, requiring minimal handling by the end user. Additionally, the readout time was reduced to 24 to 48 hours which was a significant benefit. Further advances toward the latter half of the 20th century involved the introduction of the Rapid Readout Self-Contained BIs which provided the end user with actionable results

Figure 2



within 1 to 3 hours. This technology continues to enable the end user to directly measure the lethality of the sterilization process. Figure 3 shows the components of a Rapid Readout Self-Contained BI.

Figure 3. Components of a Rapid Readout Self-Contained Biological Indicator



Is it Worth the Wait for a BI Result?

It is important to note that increased demands on faster instrument turnaround within the healthcare setting have necessitated the evolution of BIs with faster readout times. In some instances, the technological advances described in the

previous section may still not be fast enough to meet today’s healthcare requirements. Despite these limitations, the value provided by the BI however continues to be unparalleled. There have been numerous accounts of a positive BI signaling a sterilization failure that was missed by other sterilization monitors. Bryce et al. conducted an investigative study to identify the root cause of a series of positive BIs in steam autoclaves.¹² These positives occurring in sterilizers in multiple departments within the same building were successfully traced back to blocked steam traps within the building condensate lines, leading to an air/steam mixture. In this investigation it is clearly evident that the information provided by the BI was instrumental to identifying the cause of the ineffective sterilization process.

Other studies such as those described by Rutala et al.¹³, Gurevich et al.¹⁴, and Schneider et al.,¹⁵ involving a side-by-side comparison of different types of monitors for sterilization efficacy demonstrated the superiority of spores in being able to detect failures that were often missed by the other monitors. For example, in the Schneider study only the BIs (both conventional and enzyme-based early-readout) consistently detected the suboptimal steam sterilization failures related to superheat and incomplete air removal. The chemical indicators tested, which included products now labeled as Class 5 Integrating Indicators and Class 6 Emulating Indicators, failed to detect these steam sterilization failures by showing an accept or pass result.

At the Association of Professionals in Infection Prevention (APIC) 2008 Annual meeting in Denver, Dr. William Rutala noted the following in his seminar entitled

Disinfection & Sterilization: Current Issues & New Technologies.¹⁶

“No professional organization (e.g., AORN) has recommended the use of Class 6 emulating indicators as a substitute for biological indicators and there are no data that demonstrate that it mimics a BI at suboptimal sterilization times.”

Dr. Rutala recommends the following which is aligned with AAMI ST79 and AORN recommended practice:

- ▶ “Monitor each load with physical and chemical (internal and external) indicators. If the internal indicator is visible, an external indicator is not needed.
- ▶ Use biological indicators to monitor effectiveness of sterilizers at least weekly with spores intended for the type of sterilizer (Class 6 emulating indicators are not a substitute).
- ▶ Use biological indicators for every load containing implantable items and quarantine items, whenever possible, until the biological indicator is negative.”¹⁶

These and other studies have repeatedly demonstrated the unique ability of BIs to pick up sterilization process failures due to causes such as superheated steam, air/steam mixtures and inadequate air removal which are not detected by other currently available monitors such as physical monitors and Class 1-6 Chemical Indicators (CIs). An expert, Dr. Rutala also noted this in his presentation at APIC, 2008.

PRACTICAL APPLICATION

- Yes, it is worth the wait for the BI results because of their unique ability to detect superheated steam, air/steam mixtures and inadequate air removal.

Important Sterilization Concepts

Having reviewed the evolution of BIs and the role they play in monitoring the effectiveness of the sterilization process, the sections below describe important scientific concepts in sterilization.

Choice of resistant microorganisms

Microorganisms vary in resistance with vegetative forms of bacteria and fungi being the least resistant, followed by large viruses, small viruses and ultimately bacterial spores showing the highest resistance. The choice of a suitable reference organism for testing sterilization process effectiveness involves utilizing these inherent resistance properties with spores of *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) for moist heat and certain strains of *Bacillus atrophaeus* (formerly *Bacillus subtilis*) for ethylene oxide sterilization.

What is a survivor curve?

A survivor curve, also known as a death rate curve, is a graphic representation of microbial inactivation over increased exposure to stated conditions.²

What is survival/kill?

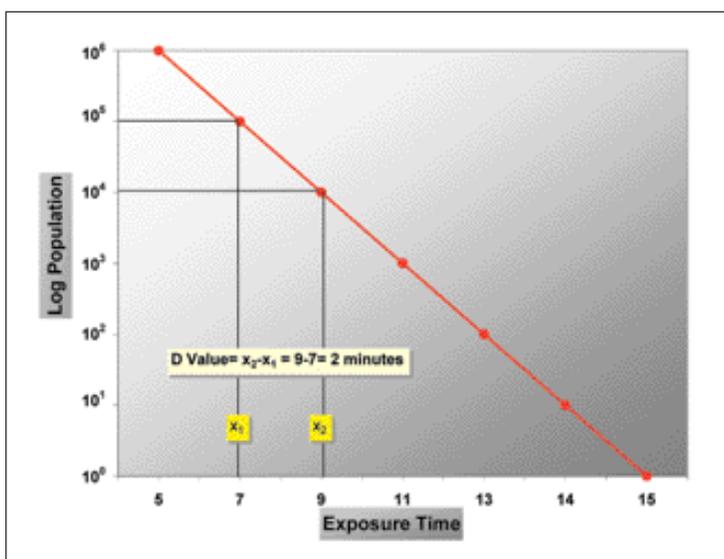
Biological indicators have to be designed to meet certain specifications regarding a time point at which all spores need to survive (referred to as survival) and a time point at which all spores need to be inactivated (referred to as kill). The formulae given below are used to calculate typical survival/kill time points.

- ▶ Minimum Expected Survival Time
Survival Time = Not Less Than $D - \text{value} \times (\log_{10} \text{ viable spore population} - 2)$
- ▶ Maximum Expected Kill Time
Kill Time = Not More Than $D - \text{value} \times (\log_{10} \text{ viable spore population} + 4)$

What is D-Value

When a microbial population is subjected to sterilization, the rate of death of individual spores is linear when the logarithmic numbers of

Figure 4. Concept of D-Value



survivors is plotted against time. The resistance of an organism that governs this logarithmic decrease is defined by the concept of D-value. In other words, the D-value is the time taken at a fixed temperature to achieve a 90 percent (or 1 log) reduction in viable cells (see Figure 4 on page 86). The D-value calculation assumes a linear decrease in population. For example, if a BI containing a population of 1×10^6 has a D-value of 2 min at 121°C it means that 90 percent of the population will be killed within the first 2 minutes of the cycle. During the next 2 min, 90 percent of what was left behind is killed and this pattern continues progressively in a logarithmic manner until no survivors can be recovered. "The higher the D-value, the more resistant the microorganism is to destruction."²

What is z-value?

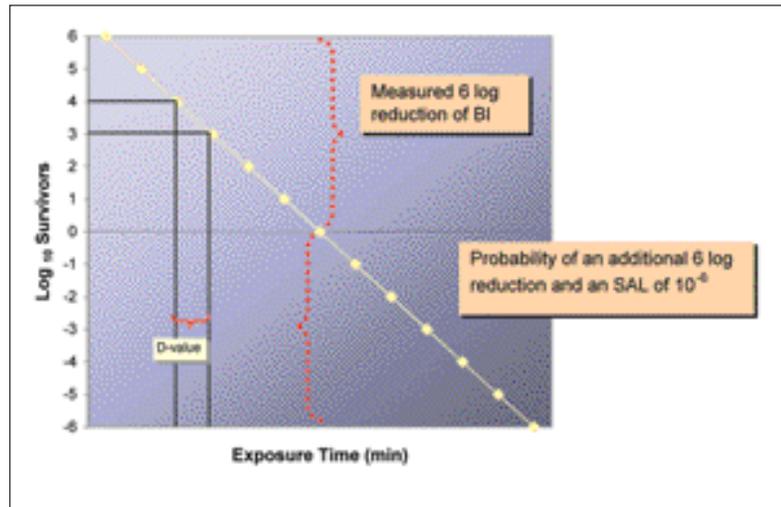
Z-value is the temperature increase required to reduce the thermal death time or D-value by a factor of 10 (or a one-cycle).

What is SAL?

Sterility is defined as being free from all living organisms.¹⁰ Since it is not practical to test every device for the absence of microorganisms, the concept of sterility is assumed to be a statistical probability. This concept defines an assurance level commonly referred to as the sterilization assurance level or SAL for validated sterilization processes. SAL is defined as the \log_{10} number of the probability of a survivor on a single unit or device. If the probability of finding a surviving organism is one in a million, then the SAL would be 10^{-6} . Using the concept of D-value we reviewed in the previous section, the microbial population can be expected to decrease progressively during the sterilization process. In order to achieve an SAL of 10^{-6} , the process is continued until a probability of attaining one surviving organism in a million is achieved. Assuming a validated process, this SAL of 10^{-6} (Figure 5) is an accepted requirement for a product labeled as sterile.

Given that the number of resistant spores contained in a BI is far greater than the routinely encountered microbial challenge on medical devices, a negative BI result indicates that the process delivered sufficient lethality. This ensures a built-in margin of safety inherent to the BI.

Figure 5. Survivor Curve Representing an SAL of 10^{-6}



PRACTICAL APPLICATION

- The BIs chosen should contain the most resistant spore for the sterilization process being tested.
- The higher the D-value the more resistant the BI.
- BIs provide the margin of safety needed to ensure the efficacy of a sterilization process and no additional monitor is needed (i.e., Chemical Indicator) to add any more safety margin.

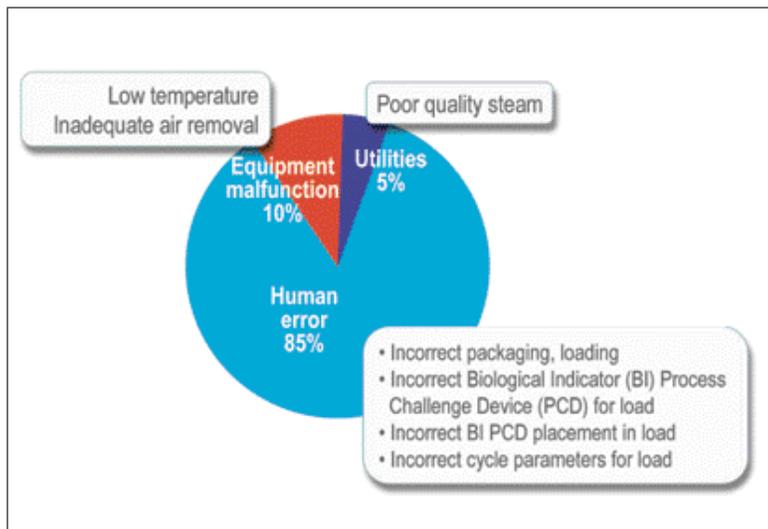
Steam Sterilization Process Failures

Human error is the main cause of steam sterilization process failures, followed by equipment malfunctions and steam quality (see Figure 6).¹⁷ The sterilization process failures that result are due to inadequate air removal, superheated steam or steam quality issues and/or inadequate time at temperature.

Why is adequate air removal important for effective sterilization?

The presence of air in the chamber (referred to as non-condensable gas) during the sterilization process hinders the optimal diffusion of steam. In the absence of air, the steam condenses upon coming in contact with a cooler object and effectively transfers heat and inactivates any microbial bioburden. When air is present, however, this condensation is impaired and the effectiveness of steam is greatly reduced. Additionally the poor mixing properties of steam and air result in temperature variations throughout the sterilization chamber. **Small changes in temperature can reduce the margin of safety with steam processing** (see Tables 2 and 3 on page 90).

Figure 6. Variables Affecting the Outcome of Steam Sterilization



When residual air is present in the sterilization chamber, air pockets are formed within the load, which are impermeable to steam thus greatly reducing the efficacy of the sterilization process. This problem can be worsened in the presence of loads containing porous packs, pouches or containers as the air is attracted towards these locations and not displaced easily. Most sterilizers possess some form of automatic air removal capability (dynamic-air-removal sterilizers). Based on the sterilizer air removal efficiency, the steam diffuses into the free spaces throughout the load and effectively results in destruction of the microbial life forms. Rapid and complete air evacuation is achieved by thermostatic valves normally referred to as steam traps. It is important to note that most valves have a use life beyond which they deteriorate therefore requiring the end user to check their proper functioning on a regular basis.² The use of BIs is one way to consistently detect the presence of air.¹⁵

Why is steam quality important for effective sterilization?

Moist heat is considered to be one of the most effective sterilization methods. In order for the steam to be completely effective, however, the quality of steam plays an important role. When steam is completely saturated it is able to efficiently transfer the required heat for the inactivation of microorganisms. Occasionally one hears the term superheated steam, also known as “dry steam.” In this state, the water content of the steam is less than that of saturated steam which in turn greatly impacts the sterilization process efficacy.

This phenomenon, which can be caused by sterilizer conditions such as inadequate steam boilers and heat radiated by the chamber jacket, or load conditions such as tightly loaded packs or the presence of dry absorbent materials, tends to go unnoticed. **As seen with inadequate air removal, steam quality issues will also result in changes in the temperature affecting the “time at temperature kinetics” thereby reducing the efficacy of the sterilization process.** Schneider et al. performed a side-by-side comparison of different sterilization monitors in superheated steam condi-

tions demonstrating that the only monitor that was able to detect this failure condition was a BI.¹⁵

Why are small changes in temperature important?

Steam sterilization principles are based on the relationship of processing time (t) versus temperature (T) as a logarithmic function. Under conditions of saturated steam, the independent variables of time and temperature can be determined by the following formula:¹⁸

$$t = F_0 \times 10^{(250-T)/z}$$

t = time for 100% kill at temperature T

T = processing temperature

F₀ = Kill time for *G. Stearothermophilus* with a z = 18°F (10°C) and a D-value of 1 minute at 250°F (121°C)

z = rise in temperature required to increase the rate of kill by a factor of 10 (usually about 18°F or 10°C)

In the previous sections, we have reviewed the potential causes for inadequate temperatures within the sterilization loads. We can use this formula to understand the impact of small changes in temperature on the time it takes for 100 percent kill of *G. stearothermophilus* spores in a BI.

For this example, let us assume that it takes **12.0 minutes** to kill **1 million** living spores, with a z value of 18°F (10°C) in a 250°F (121°C) steam sterilization process. So, if the actual temperature was 248°F (120°C), instead of the desired 250°F (121°C), there would be a corresponding increase in the time required for 100 percent kill (as indicated by a F₀ value of 12 min) as follows:

$$t = (12.0)10^{(250-248)/18}$$

Where

F₀ = 12.0 min for *G. stearothermophilus*

z = 18°F (10°C) for *G. stearothermophilus*

In order to show the high sensitivity of kill time to temperature, the above formula can be solved for 248°F (120°C).

$$t = (12.0)10^{(250-248)/18}$$

$$t = (12.0)10^{(0.11)} = (12.0)(1.29)$$

$$t = 15.49 \text{ minutes}$$

In theory, therefore, if the inside temperature of a sterilizer were actually operating at 248°F

(120°C) instead of 250°F (121°C) or the temperature inside the packs was only at 248°F (120°C) instead of 250°F (121°C), a time of **15.49 minutes** would be required to kill the **1 million** living spores of *G. stearothermophilus* at 248°F (120°C) versus the 12.0 minutes needed to kill the spores at 250°F (121°C). Table 2 provides further examples of the impact of small and large temperature changes on the time to achieve 100 percent kill in this particular case.

Table 2. Effect of deviations in temperature from 250°F (121°C) on time to achieve 100% kill of *G. stearothermophilus* spores with an F₀ value of 12 minutes

Temperature (°F)(°C)	Time for 100% Kill	Sterilization Condition
250°F (121°C)	12.0 min	Optimal condition
249.5°F (120.8°C)	12.79 min	Sub-optimal-Small deviation in temperature
248°F (120°C)	15.49 min	Sub-optimal-Small deviation in temperature
245°F (118°C)	22.75 min	Sub-optimal-Large deviation in temperature
240°F (115.6°C)	43.13 min	Sub-optimal-Large deviation in temperature

A similar exercise can be conducted with sterilization cycles at 270°F (132°C), another commonly used temperature for sterilization. For this purpose let us assume the following:

It takes **0.8 minutes** to kill **1 million** living spores with a z-value of 18°F (10°C) in a 270°F (132°C) steam sterilization process. So, if the actual temperature was 268°F (131°C) instead of the desired 270°F (132°C), there would be a corresponding increase in the time required for 100% kill (as indicated by a F value of 0.8 min) as follows:

$$t = (0.8)10^{(270-268)/18}$$

Where

F = 0.8 min for *G. stearothermophilus*

z = 18°F (10°C) for *G. stearothermophilus*

In order to show the high sensitivity of kill time to temperature, the above formula can once again be solved for 268°F (131°C).

$$t = (0.8)10^{(270-268)/18}$$

$$t = (0.8)10^{(0.11)} = (0.8)(1.29)$$

t = 1.03 min

In theory, therefore, if the inside temperature of a sterilizer were actually operating at 268°F (131°C) instead of

270°F (132°C) or the temperature inside the packs was only at 268°F (131°C) instead of 270°F (132°C), a time of **1.03 minutes** would be required to kill the **1 million** living spores of *G. stearothermophilus* at 268°F (131°C) versus the 0.8 minutes needed to kill the spores at 270°F (132°C). Table 3 provides further examples of the impact of small and large temperature changes on the time to achieve 100 percent kill in this particular case.

Table 3. Effect of deviations in temperature from 270°F (132°C) on time to achieve 100% kill of *G. stearothermophilus* spores with a F value of 0.8 minutes

Temperature (°F)(°C)	Time for 100% Kill	Sterilization Condition
270°F (132°C)	0.8 min	Optimal condition
269.5°F (131.9°C)	0.85 min	Sub-optimal-Small deviation in temperature
268°F (131°C)	1.03 min	Sub-optimal-Small deviation in temperature
265°F (129°C)	1.52 min	Sub-optimal-Large deviation in temperature
260°F (126.7°C)	2.88 min	Sub-optimal-Large deviation in temperature

This interdependence of time and temperature (in the presence of saturated steam) is an important relationship which should be understood by all personnel responsible for providing sterility assurance for steam sterilized items. Failure to achieve adequate temperature may be a realistic occurrence if the load were processed at a suboptimal temperature as a result of a minor malfunction of the sterilizer (e.g., air pocket or small air leak), a slight calibration error in the temperature monitoring system, poor steam quality, or human errors such as improper loading and packaging effects, or choosing the incorrect cycle for the load.

PRACTICAL APPLICATION

- *Steam sterilization process failures can be the result of human error, equipment malfunctions and utility problems which lead to:*
 - > Inadequate air removal in the chamber and/or the packs;
 - > Poor steam quality;
 - > Small changes of temperature in the chamber and/or the packs.
- *BIs consistently detect these failures better than all classes of chemical indicators.*

Biological Indicator Design

Biological indicators are regulated as Class II Medical Devices by the FDA and require pre-market notification (510[k] clearance) to be legally marketed.¹⁹ These devices consist of a known number of microorganisms, with a known resistance to the mode of sterilization, placed in or on a carrier and enclosed in a protective package. A negative BI result when incubated under suitable conditions along with a positive BI control indicates the efficacy of a sterilization process.

Table 4 lists the minimum populations and resistance characteristics recommended by the FDA that manufacturers need to incorporate into their BI design. Additionally, manufacturers attempt to design the BI to incorporate other functionalities that may benefit the end user such as self contained activation, reduced incubation time an automated reader/incubator, etc.

Table 4. Recommended minimum populations and resistance characteristics of biological indicators

Sterilization Cycle	Minimum Viable Spore Population	D-Value	Z-Value	Survival Time
Steam 121°C (250°F)	10 ⁵	1.5 min.	10°C	5 min.
Steam 132°C (270°F)	10 ⁵	10 sec.	10°C	1 min.
Steam 134°C (273°F) or 135°C (275°F)	10 ⁵	8 sec.	10°C	40 sec.
Ethylene Oxide 600mg/L, 60%RH, 54°C (129°F)	10 ⁶	3 min.	Not applicable	15 min.
Dry Heat 160°C (320°F)	10 ⁶	3 min.	Not applicable	12 min.

PRACTICAL APPLICATION

- *The minimum spore population for a steam BI should be 1 X 10⁵ spores/strip.*

Use of Biological Indicators

By sterilizer and medical device manufacturers

Commercially available BIs are designed to provide a challenge to the sterilization process that exceeds the challenge of the natural bioburden in or on the product. A commonly used approach to validate a full sterilization exposure time that is used by sterilizer and medical device manufactures is the use of a method called a half cycle “overkill” method.²⁰ Typically, this validation is achieved by completing three consecutive successful half cycles (i.e., cycles shortened to half the normal time) in order to qualify the proposed exposure time for routine sterilization processing of medical devices. Success is defined by the inactivation of all BIs after incubation for all the three half cycles tested. This means that if there are no positive BIs for three test cycles at 4 minutes exposure at 250°F (121°C), then a routine exposure time of 8 minutes at the same temperature would be an adequate sterilization process for the medical devices. Since half cycle testing is conducted using the worst case

validation test load the final exposure time is aptly named an “overkill” method.

Based on this rationale, it is perfectly normal for a BI to be inactivated at half the stipulated exposure time since that is what it was designed to do. As a true measure of lethality, the BI can be depended upon to detect a failed sterilization process if there was a gross or even marginal failure such as the inability to reach temperature, inadequate air removal, superheated steam, etc. described elsewhere in this article. This ability to represent a direct killing of the most resistant form of microbial life is what gives the BI the unique position of a “Gold Standard.”

By healthcare facilities

AAMI ST79 states:

“The use of BIs provides evidence of efficacy by challenging the sterilizer with a large number of highly resistant bacterial spores. Biological monitoring provides the only direct measure of the lethality of a sterilization cycle.”¹⁰

This is why biological indicators are used within a PCD (user assembled or commercially available, disposable or preassembled process challenge device, formerly called test or challenge pack):¹⁰

- ▶ to routinely monitor sterilizers at least weekly, but **preferably every day** that the sterilizer is in use in each type of cycle for which a sterilizer is designed to be used:*
- gravity-displacement at 132°C to 135°C [270°F to 275°F];
- gravity-displacement at 121°C [250°F];
- dynamic-air removal at 132°C to 135°C [270°F to 275°F];
- flash at 132°C to 135°C [270°F to 275°F];
- flash with single wrapper or other packaging.

*NOTE: If a sterilizer will run the same type of cycle (e.g., dynamic-air-removal at 132°C to 135°C [270°F to 275°F] for different exposure times (e.g., 4 minutes and 10 minutes), then only the shortest cycle time needs to be tested.

For flash sterilization each type of tray configuration in routine use should be tested separately.

- ▶ for sterilizer qualification testing after:
 - installation;
 - relocation;
 - malfunctions;
 - major repairs;
 - and sterilization process failures.

- ▶ **to monitor ever load containing implants which should be quarantined until the BI is negative.**

In addition, a BI should also be used for periodic quality assurance testing of representative samples of actual products being sterilized. When a positive BI occurs AORN and AAMI both state a recall should be initiated. AAMI ST79 states:

“If it is determined that the sterilization failure was not the result of operator error (e.g., selection of the incorrect cycle), items processed in that sterilizer since the last negative BI results should be considered unsterile. They should be retrieved, if possible, and reprocessed. The sterilizer in question should be taken out of service.”¹⁰

PRACTICAL APPLICATION

- *The advantage of running a BI PCD in each load is to ensure all implants are monitored and quarantined until the BI is negative, to avoid recalls, and to ensure that all sterilization modes used for all sterilizers and all types of packaging used in flash sterilizers are routinely tested.*

Summary

Biological indicators continue to be the “**Gold Standard**” for monitoring the sterilization process because of their ability to provide a direct measure of the lethality or the ability of the sterilization process to kill spores. It is worth the wait for the biological indicator result because of their unique ability to detect sterilization process failures due to superheated steam, air/steam mixtures and inadequate air removal which are not consistently detected by physical monitors or Class 1-6 Chemical Indicators. Remember, the objective of a sterilization process is to deliver sufficient lethality to destroy or kill the bioburden on the medical devices which is not measured by physical monitors or the end point change of a chemical indicator. No additional margin of safety is needed beyond what the biological indicator can provide.

Biological indicators continue to be the “**Gold Standard**” for monitoring the sterilization process because of their ability to provide a direct measure of the lethality or the ability of the sterilization process to kill spores.

Definitions

- ▶ **Sterilization:** Validated process used to render a product free of all forms of viable microorganisms.¹⁰
- ▶ **Sterile:** Free from viable microorganisms.¹⁰
- ▶ **Biological Indicator (BI):** Microbiological test system providing a defined resistance to a specified sterilization process.¹⁰
- ▶ **Class 5 Integrating Indicators:** Chemical indicator designed to react to all critical variables, with the stated values having been generated to be equivalent to, or exceed, the performance requirements given in the ISO 11138 series for BIs.¹⁰
- ▶ **Class 6 Emulating Indicators:** Chemical indicator that is a cycle verification indicator designed to react to all critical variables of a specified sterilization cycle, with the stated values having been generated from the critical variables of the specified sterilization process.¹⁰
- ▶ **D-value:** Time or dose required to achieve inactivation of 90 percent of a population of a test microorganism under stated exposure conditions.²
- ▶ **z-value:** Temperature change required to effect a 10 fold change in D values.²
- ▶ **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).¹⁰
- ▶ **Sterility Assurance Level (SAL):** Probability of a single viable microorganism occurring on product after sterilization.¹⁰
- ▶ **Survivor Curve:** The graphic representation of inactivation against increasing exposure to stated conditions.² †

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For additional information regarding Certification contact: CBSPD, 121 State Hwy 31N, Suite 500, Flemington, NJ 08822 or call 908-788-3847 or visit the Web site at www.sterileprocessing.org.

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