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Positive biological indicators

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LEARNING OBJECTIVES

1. *Identify the performance characteristics of a biological indicator.*
2. *Explain how Rapid Readout biological indicators detect sterilization process failures.*
3. *Discuss what a marginal sterilization process is.*
4. *Develop policy and procedures for the appropriate use of biological indicators.*

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Positive biological indicators (BI) set in motion the recall of all medical devices processed since the last negative BI, an analysis of what caused the failure, correction of those causes, and retesting of the sterilizer before it is put back into routine use.[1] The purpose of the BI is to identify when microorganisms are not killed, which is a sterilization process failure. So, when you get a positive BI the appropriate question to ask is: what changed or was different about this sterilization process that the microorganisms were not killed?

Definition and performance characteristics of biological indicators

The Association for the Advancement of Medical Instrumentation (AAMI) defines a biological indicator as a "device intended for use by a health care provider to accompany products being sterilized through a sterilization procedure and to monitor adequacy of sterilization. The device consists of a known number of microorganisms, of known resistance to the mode of sterilization, in or on a carrier and enclosed in a protective package. Subsequent growth or failure of the microorganisms to grow under suitable conditions indicates the adequacy of sterilization."[1]

A BI consists of a calibrated population of bacterial spores of a high resistance to the mode of sterilization being monitored. For example, Geobacillus stearothermophilus is the most resistant spore for steam sterilization, hydrogen peroxide gas plasma and ozone sterilization. Bacillus atrophaeus is the most resistant spore for ethylene oxide (EO) sterilization. In some BI configurations, spores are coated on a carrier, which is enclosed in a plastic vial containing a crushable glass media ampoule and cap that allows the sterilant to penetrate into the plastic vial, killing the spores and demonstrating whether sterilization conditions were met. This is called a self-contained biological indicator; Figure 1, below, shows the components of an exemplary BI.

The performance characteristics of a BI are defined in the Association for the Advancement of Medical instrumentation (AAMI) standards.[2,3] BI performance is based on spore population, D-value and survival/kill values. See Table 1 (next page) for an example of BI performance data for steam sterilization and Table 2 (next page) for BI performance data for EO sterilization. This data is included in a Quality Assurance Certification that is found in each box of product.

The labeling of a BI will state which sterilization cycle the BI can be used for, which spore is contained on the carrier, and what the population of the spores is. The population is expressed as the mean number of spores per strip and the term colony forming unit (CFU) is used. If the population is listed as 3.7x106 CFU, there are 3,700,000 spores on the carrier. In order to be an appropriate challenge for the sterilization process, the population of spores must not be less than 1x106 CFU for EO sterilization processes and 1x105 CFU for steam sterilization processes.[2,3] A BI with a spore count less than these would not be considered an appropriate challenge.

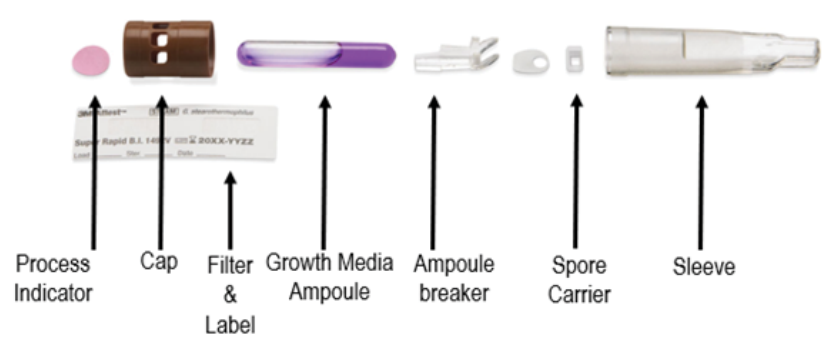

Figure 1. Brealout of biological indicator

Table 1. Biological indicator performance data for stream sterilization processes.	
For use in monitoring the 250°F (121°C), gravity and 270°F (132°C) vacuum assisted steam sterilization process.	
Organism: Geobacillus stearothermophilus ATCC 7953	
Population (mean/strip): 3.7x106 CFU	Determined at time of manufacture. Population is reproducible only under the exact conditions under which it was determined.
Resistance Testing Data	
Test D-value (121°C): 1.6 minutes	Survival/kill is verified and D-value is determined in a BIER vessel using a gravity cycle. D-values are determined by a fraction negative procedure after graded exposures to sterilization conditions. D-value is reproducible only under the exact conditions under which it is determined. User would not necessarily obtain the same results and would need to determine the biological indicators suitability for their particular use.
Survival time (121°C): 7.3 minutes	
Kill time (121°C): 16.9 minutes	

The D-value is defined as the decimal reduction value, which indicates the resistance of the BI. The larger the D value, the more resistant the microorganism is to destruction.1 D-value testing is determined in a biological indicator evaluation resistometer (BIER) test vessel that has a small chamber, no come-up-time or load. For BIs used for steam sterilization, this testing is done at 121°C (250°F). The D-value is the exposure time required to secure inactivation of 90 percent of a population of test organisms under stated conditions. For example, if a BI used for steam sterilization states that the D-value (121°C) is 1.6 minutes, it means 90 percent of the spore population is killed in the first 1.6 minutes of a 121°C steam sterilization cycle. During the next 1.6 minutes, 90 percent of the remaining spore population is killed. By this data, you can tell that all spores do not die at the same time. There is a transition period between all spores surviving and all spores being killed. During this transition period, when some negative and some positive BIs are obtained, the cycle is described as consisting of marginal sterilization conditions.

Biological indicator performance is also defined by survival/kill values. This also relates to the resistance of the biological indicator. The survival time is the time at which all spores in the BI will still be alive. The kill time is the time at which all spores in the BI will be killed. The survival and kill value can be determined by testing in a BIER test vessel or can be calculated based on the spore count and the D-value. BI performance data should be included in each package of product, usually in a Certificate of Analysis.

Evolution of biological indicators

In the 1970s, self-contained BIs first became commercially available. Self-contained BIs had three major advantages over multicomponent BIs. First, they eliminated the need to aseptically transfer the spore strip to a liquid growth media by combining the spore strip (or carrier) and a crushable glass ampoule in the same container. This addressed the common contamination problem of spore strips. Second, the addition of a pH dye, which turned yellow when microbial growth produced acidic by-products, was used to detect positives in place of observing for cloudy media indicating microbial growth. This greatly simplified interpretation of the results and put BI testing in the hands of the sterilization departments rather than the microbiology laboratory. The third advantage is faster read-out times. As refinements in recovery media were developed, they resulted in shorter required incubation times. These advantages have resulted in the elimination of spore strips that require aseptic transfer to media and incubation wherever possible. These advantages, plus labor and time savings, have resulted in the widespread use of self-contained BIs.

The need to verify the efficacy of the sterilization process in a shorter time period has been becoming more important because of the turnover demands on the sterilization department, the complexity of medical devices being introduced, and the need to save time and control costs. These needs drove the development of rapid readout BIs. Rapid readout BIs (indicators with enzyme-based early-readout capability) are identical to the original self-contained BIs with one major exception: The glucose in the media has been removed and replaced by a glucoside (or equivalent glucosidase substrate) with a fluorescent indicator dye attached. Spores that have not been destroyed by a sterilization process and are biologically active are demonstrated in a much shorter period of time because, as soon as the glucoside is broken down, the fluorescent dye becomes detectable in trace amounts. Spores do not need to multiply to release the dye from the glucose substrate. A proper sterilization process will sufficiently destroy cellular components so that microbes are no longer able to grow. Following a proper sterilization process, neither detectable enzymatic activity is present nor is the cell able to grow or multiply. An auto-reader detects the presence of the naturally occurring enzyme, which is an intrinsic component produced by the

Table 2. Biological indicator performance data for ethylene oxide sterilization processes.	
For use in monitoring the ethylene oxide sterilization process.	
Organism: Bacilus atrophaeus ATCC 9372	
Population (mean/strip): 3.9x106 CFU	Determined at time of manufacture. Population is reproducible only under the exact conditions under which it was determined.
Resistance Testing Data	
Test D-value (121°C): 3.4 minutes	Survival/kill is verified and D-value is determined in a BIER vessel at 54°C, 60 percent RH, 600 mg ethylene oxide/liter. D-values are determined by a fraction negative procedure after graded exposures to sterilization conditions. D-value is reproducible only under the exact conditions under which it is determined. User would not necessarily obtain the same results and would need to determine the biological indicators suitability for their particular use.
Survival time (121°C): 15.99 minutes	
Kill time (121°C): 36.99 minutes	

Self-Study Test Answers:1. D, 2.C, 3. B, 4. D, 5.B, 6. B, 7. D, 8. A, 9. A, 10. B

spore, by reading a fluorescent product that is produced when this enzyme converts the non-fluorescent substrate in the media vial.[4] The fluorescence indicates the presence of an active enzyme and a sterilization process failure. Non-fluorescence indicates inactivation of the enzyme and an effective sterilization process. A sterilization process failure can be detected in as little as a few minutes, which is an improvement over the one to seven days previously required.[5] Obtaining results within a minimal incubation time allows sterilization process failures to be identified much sooner, instruments to be turned around faster, costs associated with inventory and recall to be reduced, and improved patient outcomes.[6]

When a biological indicator is doing its job

BIs detect conditions that are not able to kill the spores. Since spores are more resistant than other microbes, they provide a safety margin. If the spores have been killed, then by inference, the other microbes on medical devices should have also been killed. Sterilization cycles are designed to kill spores within the first half of the exposure cycle. In a normally functioning cycle, the spores should easily be destroyed. See Figure 2, below, for a graphic representation of spore kill in a typical sterilization process.

At the beginning of the process, all spores are expected to be alive. By the middle of the process all spores should be killed. At some point between these two states, marginal cycle conditions exist. A marginal cycle is one that fails to completely kill all spores, and can yield both positive and negative BI results. In a sterilization process failure (i.e., sterilizer not functioning, inadequate steam quality and quantity, or human errors due to incorrect packaging, loading, or choosing the incorrect cycle for the load) the marginal part of the process may come at the end of the cycle. It is toward the end of these marginal cycle conditions where a more sensitive indicator, such as a fluorescent dye, may detect a few more positives than a less-sensitive indicator, such as a pH dye. Detection of biologically active proteins, such as the intrinsic enzyme in the spore that breaks down the glucoside substrate containing the fluorescent dye, demonstrates a sterilization process failure. Whether the spore is able to multiply or not, the detection of biologically active proteins demonstrates a sterilization failure.

Rapid readout BIs can detect marginal cycle conditions that other spore strips and self-contained BIs do not. Vesley and Allwood concluded in their evaluation of BIs that rapid readout BI technology was a more sensitive indicator of marginal sterilization cycles than other self-contained BIs without any indication of false positive results.[7] Likewise, Rutala et al. reported that rapid readout BIs were a suitable monitor that ensures sterilization without inappropriately indicating failure.[8]

Recommended practices for using biological indicators

A process challenge device (PCD) is a test pack that creates a challenge to the sterilization process that is greater than or equal to a routinely processed item.[9] The AAMI and the Association for the periOperative Registered Nurses (AORN) recommend that a BI in a PCD be run weekly, but preferably every day that the steam sterilizer is used.[1,10] Additionally BIs within PCDs should be used for sterilizer testing after sterilizer installation, relocation, malfunction or process failure, and any major repairs.[1,9] This testing should be done in each type of cycle (gravity-displacement, dynamic air-removal [prevaccum or steam flush pressure pulse]) used. If a sterilizer runs cycles for different exposure times, then the shortest cycle time should be tested.[1] In addition, when using the immediate-use-steam-sterilization (IUSS) sterilization process, each type of tray configuration (e.g., open surgical tray, single-wrapped surgical tray, protective organizing case, rigid sterilization container) in routine use should be tested separately. Each load containing implantable medical devices should be monitored with a PCD containing a BI and a Type 5 integrating chemical indicator, and the implantable device quarantined until the results of the BI testing are available. If a PCD containing only a BI is used to release a sterilized load, it should be quarantined until the BI results are known.[1]

BIs are used for qualification testing by the sterilizer manufacturer at time of installation, and by the healthcare facility for periodic quality assurance testing. BIs are also used for product testing.[1]

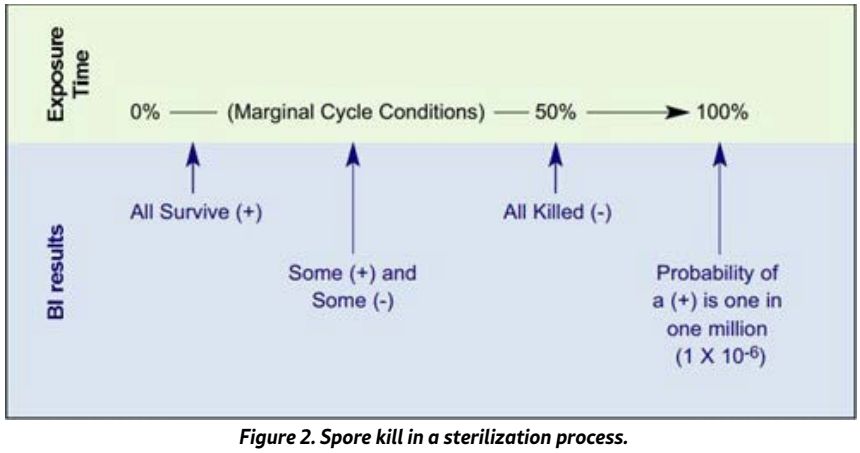

Figure 2. Spore kill in a sterilization process.

Summary

Biological indicators (BIs) provide direct evidence that the sterilization process conditions are able to kill spores. BIs have evolved over the past 50 years. Results that once took seven days or more now are obtained in less than 1 hour, and less than 30 minutes in some cases. Cumbersome subculturing and long incubation times have been replaced by self-contained biological indicators with rapid readout techniques. HPN

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Positive biological indicators

Circle the one correct answer:

1. **Which of the below statements about biological indicators (BI) is/are true?**
   A. A positive BI result should be investigated to determine the cause.
   B. The purpose of a BI is to identify when microorganisms are not killed.
   C. A BI contains a known number of microorganisms.
   D. All of the above.

2. **Which of the below measures do NOT contribute to the performance characteristics of BIs?**
   A. D-values
   B. Survival and kill values
   C. Cost
   D. Spore population

3. **What does the acronym CFU stand for?**
   A. Conditions for use
   B. Colony forming unit
   C. Critical failure underkill
   D. None of the above

4. **What requirements need to be met for a BI to be an appropriate challenge for the sterilization process?**
   A. Contain microorganisms with a high resistance to the mode of sterilization being monitored
   B. Contain at least 1x106 CFU for ethylene oxide sterilization processes
   C. Contain at least 1x105 CFU for steam sterilization processes
   D. All of the above

5. **A marginal sterilization cycle ___.**
   A. successfully kills all microorganisms
   B. can yield both positive or negative BI results
   C. is never due to human error
   D. All of the above

6. **Sterilization cycles are designed to kill all spores at which point in the cycle?**
   A. 25%
   B. 50%
   C. 90%
   D. 100%

7. **Which of the following factors could contribute to a sterilization process failure?**
   A. Sterilizer malfunction
   B. Inadequate steam quality
   C. Selection of the incorrect cycle for the load
   D. All of the above

8. **Which of the following statements is/are NOT true about rapid readout BIs?**
   A. Rapid readout BIs are less reliable than slow-read BIs.
   B. Rapid readout BIs contain an enzyme substrate attached to a fluorescent indicator dye.
   C. Rapid readout BIs can detect biologically active spores without needing to wait for the spores to multiply.
   D. All of the above

9. **What is the AAMI ST79 and AORM recommended frequency for monitoring steam sterilization cycles with a BI in a process challenge device?**
   A. At least weekly, preferably daily, plus implants
   B. At least monthly, preferably weekly, plus implants
   C. Only after a sterilization process failure
   D. None of the above

10. **Which of the following statements are true about monitoring with BIs?**
    A. BI testing does not need to be done in both gravity-displacement and dynamic air-removal cycles if they are done in the same sterilizer
    B. Loads containing implantable devices should be monitored with a BI and Type 5 chemical indicator, and quarantined until the results of the BI results are available
    C. If a sterilizer runs cycles at multiple different exposure times, then a BI should be tested using the longer cycle time
    D. None of the above

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