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LEARNING OBJECTIVES

1. Describe the design and function of biological indicators.
2. Understand how rapid readout biological indicator systems work.
3. Discuss the recommended uses of biological indicators for sterilization monitoring.

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SELF-STUDY SERIES

The science of speed

Today's rapid readout biological indicators

by Craig Wallace, Senior Technical Specialist, 3M Infection Prevention Division

Biological indicators (BIs) are an important part of a quality control system for hospital sterilization processes. The information on the quality of the sterilization process supplied by biological indicators, when combined with the information from physical monitors and chemical indicators, provides the basis for the decision on whether or not to release the medical devices for use on patients.

Biological indicators are defined as a test system containing viable microorganisms providing a defined resistance to a specified sterilization process.¹ A key point in this definition is "viable microorganisms," as biological indicators are the only sterilization monitoring device that directly tests the effect of the sterilization process on microorganisms. The Centers for Disease Control and Prevention describe the value of biological indicators in their 2008 Guideline:

*"Biological indicators are recognized by most authorities as being closest to the ideal monitors of the sterilization process because they measure the sterilization process directly by using the most resistant microorganisms (i.e., Bacillus spores), and not by merely testing the physical and chemical conditions necessary for sterilization. Since the Bacillus spores used in biological indicators are more resistant and present in greater numbers than are the common microbial contaminants found on patient-care equipment, the demonstration that the biological indicator has been inactivated strongly implies that other potential pathogens in the load have been killed"*²

The primary biological indicator design used in healthcare facilities is the self-contained biological indicator, or SCBI (see Figure 1). Self-contained biological indicators contain the critical elements of the biological indicator: the bacterial spores on a carrier, and the growth media required to culture the test organisms to determine if the BI is positive or negative. The self-contained design

eliminates the need for a microbiological laboratory to complete the BI test.

Before we go much further into biological indicators we need to take a minute and review a little bit of microbiology. The term "spores" is short for bacterial endospores. There are a few types of bacteria that have developed the ability to change from an active, growing cell (or vegetative cell) to a highly protected, dormant cell (endospore), and back again depending on their environment. These bacteria will change to a spore when faced with a shortage of food or other conditions that are harmful to the cell. The spore itself is like a plant seed or hard nut – it is biologically dormant (or "sleeping"), it has a highly protective dry shell, and it is capable of withstanding extreme conditions for prolonged periods of time without ill effect. If the spore senses that conditions have improved and will support life, it goes through a series of biological steps called activation and germination, to shed the hard coat and become a regular, active bacterial cell once again. Biological indicators use the spore form of *Bacillus* bacteria because of the toughness of these spores and the challenge they present to the sterilization process. Each sterilization process requires a specific *Bacillus* species proven to be the most resistant to that process. For example, steam sterilization processes are tested with *Geobacillus stearothermophilus* spores.

Spores require a source of nutrients and optimized temperature and pH to begin the activation, germination, and outgrowth processes. Self-contained biological indicators contain growth media that has been specially formulated to support outgrowth of the spores used in that BI. All biological indicators require incubation, during which the spores are exposed to the growth media and the biological indicator is heated to the optimum temperature for spore outgrowth. Any surviving spores will first activate and germinate to become vegetative cells, and then these cells will begin to "grow," which means they will replicate (one becomes two, two become four, and so on).

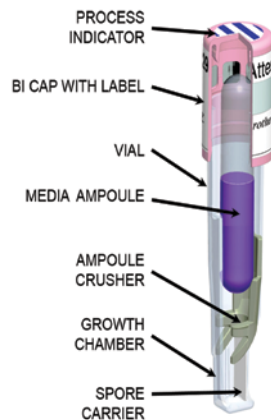
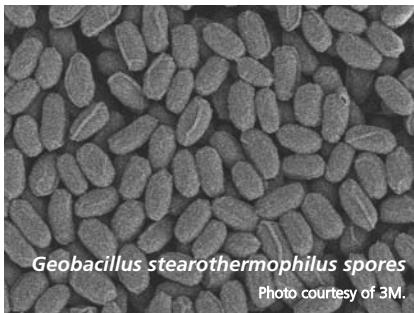


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Figure 1 – Components of a self-contained rapid readout biological indicator.



Incubation time

The incubation time for a biological indicator is the amount of time that the BI must be incubated before a decision can be made that the BI is negative (i.e., the spores are all dead) and the test is complete. This concept takes a little more explanation... if a biological indicator turns positive, it has completed its “task” of providing information on the quality of the biological indicator system (in the case of a positive control) or of the sterilization process itself (a positive BI test indicates a sterilization process failure). If a biological indicator turns positive you will end the BI test at that point and take appropriate action. But, how long must you incubate a BI before you can decide that it is truly negative and end the test? This time frame is called the incubation time.

The international biological indicator performance standards state that the reference incubation time for a biological indicator is 7 days.³ This incubation time was established in the early days of biological indicators, and was based on the technology available at that time. An incubation period of seven days is not at all practical or useful in today’s healthcare environment. So, for biological indicators, there was a need for speed.

Biological indicator evolution

The first generations of biological indicators consisted of spores applied to some sort of carrier, such as a piece of suture material. These early BIs evolved into spore strips, where the spores were applied to a small paper strip that was enclosed in a glassine envelope which allowed sterilant penetration while protecting the spore strip from outside contamination. After the sterilization process the indicators were transferred to a test tube containing the growth medium using a process called aseptic transfer. This was typically done in a microbiology laboratory equipped with special laminar flow hoods to try and prevent any environmental organisms from contaminating the spore strip or the media, which would create a false positive result. (Note: This process is not required with self-contained biological indicators). The test tubes were then

incubated at the proper temperature for up to seven days. So, how could you tell if the BI was positive or negative? The user needed to look for a “signal” from the spores that they were alive (positive BI), or dead (negative BI).

The original signal used to determine a positive or negative result was the development of turbidity, or cloudiness, in the test tube. If the BI placed in the medium had viable spores (either a positive control or a sterilization process failure), the spores would convert to vegetative cells, and begin to grow or replicate. Over time, the number of cells in the test tube would increase to the point where the density of cells in the tube was high enough to scatter light passing through the test tube, making the medium appear cloudy. While effective, this process required a significant amount of incubation time (up to 7 days) to allow the spores to germinate and the cells to grow for enough generations to be able to scatter light.

The next advance in technology introduced a color-based pH indicator into the growth media, to make the biological indicator signal a color change rather than cloudiness. A pH indicator is a chemical that responds to the acidity of the solution, and will typically be one color at an alkaline pH and change to another color as the solution becomes more acidic. Biological indicators utilizing the pH color change system have growth media that is specially formulated so that bacteria growing in the medium will produce acidic by-products. As the bacteria continue to grow, the growth medium will continue to become more acidic until the pH indicator changes color. This technology enabled the development of self-contained biological indicators in the 1970s. The glass media ampoule in the SCBI was too small to see development of turbidity, but a color change was readily apparent. The optimization of the media in SCBIs and the user’s ability to detect the color change signal faster than the cloudiness signal reduced the incubation time from 7 days to 2 days. This was much faster and easier than spore strips, but still required incubation times that were not optimal for healthcare.

The next major leap in reduction of biological indicator incubation time came from new technology that enabled detection of biological signals from viable spores much earlier in their germination and outgrowth process. To understand this, we need to understand a little more microbiology. The spore activation and germination processes may sound simple but they are actually complicated, multi-step processes. A good analogy is the steps that occur when a computer powers up. Once the

power button is pushed the computer goes through a series of actions that turn on many programs and sub-systems in the computer in a specific order, until the computer is fully operational and ready for use. In the spore, the cell’s “sub-systems” are created and activated by many biochemical reactions. Specialized proteins called enzymes act as catalysts that make these complicated reactions happen much more quickly. The first rapid readout biological indicators used the actions of some of these “boot up” enzymes to produce a signal that could be detected earlier in the spore outgrowth process, reducing the required BI incubation time from days to hours.

The enzymes used to produce a signal for rapid readout biological indicators are enzymes that become active early in the activation and germination processes. These enzymes have a specific natural role for the cell, but their catalytic actions can also be utilized to produce a signal that can be detected and analyzed as a positive response. Rapid readout biological indicator technology uses a special indicator in the growth medium that can interact with the enzyme. This chemical is like the pH indicator discussed earlier, except that instead of turning color based on a change in acidity this indicator changes from a non-fluorescent molecule to a fluorescent molecule when it is acted on by the enzyme. Fluorescence means that it will “glow” or emit light at a certain wavelength (say, Wavelength B), if it is first exposed to light of a different wavelength (Wavelength A). So, rapid readout BIs use a biological indicator reader that shines Wavelength A light onto the incubating biological indicators, and has a detector that is sensitive to Wavelength B light to look for a fluorescent signal. If the enzyme is active in the biological indicator (i.e., a positive control BI or a positive BI from a sterilization process failure), the sensors will detect the fluorescent signal and the reader analyzes this signal and indicates a positive BI result.

Rapid readout biological indicator technology has reduced incubation times from days to hours. Continued improvements of the physical design of these biological indicators concentrated the fluorescent signal to make it easier to detect. These changes, coupled with improved sensors and electronics in the readers, have now reduced biological indicator incubation times to less than an hour, and in some cases, less than 30 minutes. This dramatic improvement in incubation time, from 7 days to less than 30 minutes, means that this important quality control information regarding the efficacy of the sterilization process is now available in a timeframe that fits with the healthcare facilities’ workflow.

Quality control of sterilization processes

You can't see sterility. This basic fact drives the need for a quality control system that provides information on the quality of a sterilization process, so a decision can be made on whether or not the processed instruments are safe for patient use. The American National Standards for the key healthcare sterilization processes: steam, ethylene oxide, and vaporized hydrogen peroxide, all recommend the integrated use of three quality control monitoring tools: physical monitors, chemical indicators, and biological indicators.^{4,5,6} The information provided by each tool is different. Physical monitors are electronic sensors inside the chamber that provide data on the environment inside the sterilizer chamber such as the temperature or pressure. This data is recorded on a printout that can also be used as a record of the cycle. Review of cycle printouts from the physical monitors can confirm that the proper cycle was selected. The second quality control tool, chemical indicators, utilize specially selected chemicals that respond to the effects of the sterilization process. Chemical indicators that are used on the outside of packages (Type 1 process indicators) can provide visual evidence that an item has gone through the sterilizer. Common process indicators include indicator tapes and chemical dots printed on packages. Remember that process indicators are only designed to indicate exposure to the sterilant, and they *do not* provide evidence that the process was effective. The more sophisticated chemical indicators (Type 5 and Type 6 indicators) that are used inside of containers and packages are designed to respond to all the sterilization process variables. These chemical indicators provide more detailed information on whether the required process conditions were achieved on the inside of the packages.

As we have discussed, the third quality control tool, biological indicators, are used to directly measure the effectiveness of the sterilization process by measuring its effect on live microorganisms. Let's take a closer look at the role of biological indicators in the quality control of sterilization processes.

The role of biological indicators in quality control

Biological indicators are placed with the load inside of the sterilizer chamber in the location determined to be the most difficult to sterilize. The typical biological indicator placement location for large steam sterilizers is over the drain; for ethylene oxide sterilizers, in the center of the load, and for hydrogen peroxide sterilizers at different chamber locations

specific to the sterilizer, cycle, and load. The instructions of the sterilizer manufacturer regarding the recommended placement location for the biological indicator in their sterilizer should be followed.

Biological indicators are typically used inside of process challenge devices (PCDs) or other items that can represent the sterilizer load. Placement of biological indicators inside of the containers or packages would give direct information on the lethality of the sterilization process inside the device packaging, but this placement is not practical as even today's rapid readout biological indicators require incubation time that would not be feasible in the OR. So, biological indicators are placed into PCDs or other devices that are intended to have the biological indicator perform as if it was placed inside of containers or packages in the load. Reference PCDs that can be constructed in healthcare facilities are described in the standards.^{4,5} Commercially available PCDs that have been cleared by the Food and Drug Administration (FDA) with performance equivalent to the reference PCDs are also available. These devices eliminate the need for staff time to assemble test packs, and are typically more consistent because of automated assembly processes and quality control procedures required of medical device manufacturers.

The recommended frequency of use of biological indicators in healthcare facilities varies by the sterilization process. For steam, the recommendation from AAMI is weekly use, but preferably daily use, for routine efficacy monitoring. Also, a biological indicator should be used to release any load containing an implantable device. Implant-loads should be quarantined until the biological indicator results are available.⁴ Per AAMI, a biological indicator should be used to monitor every load for ethylene oxide sterilization processes. Again, any implants should be quarantined until the biological indicator results are available.⁵ Finally, for vaporized hydrogen peroxide processes, the AAMI recommendation is that biological indicators be used daily, but preferably in every load. The same requirement of BI monitoring with load quarantine until the BI results are available is applied for implants.⁶

As you can see, there is some variability in the current recommended practices regarding frequency of use of biological indicators. It is interesting to note that this type of variation is not allowed for medical device manufacturers that are supplying sterile, single-use devices to healthcare facilities. National and international standards for medical device manufacturers require the same level of quality control

for every sterilization load, regardless of the device, intended use, day of the week, etc.^{7,8,9} It is curious that reusable medical devices reprocessed in a healthcare facility do not have to meet the same level of safety as those sterile devices received directly from manufacturers. Many healthcare facilities are now leveraging the significant reductions in biological indicator incubation time to increase their frequency of use of this important QC tool, without negatively affecting their work flow. For example, rapid readout BIs make the quarantine of implantable devices until the biological indicator test result is available much more realistic. Many hospitals have moved to monitoring of every sterilization load with biological indicators even where the current healthcare standards do not require it, such as for steam and vaporized hydrogen peroxide. Load items from these processes are not distributed until a negative BI result is obtained. The criteria often cited for making this change include a desire to improve quality control to assure a uniform standard of care for all patients, avoidance of the extra work required in the event of a recall, as well as reduction of errors in the sterile processing department caused by varying requirements for biological indicator monitoring.

Summary

Biological indicator technology has continued to evolve with faster detection of the biological signals produced by the bacterial spores that provide the direct challenge to the sterilization process. These technologies have resulted in biological indicators with incubation times of less than 30 minutes for some sterilization processes. These short incubation times now make it possible to obtain biological test results in time for optimized instrument workflow, including shorter quarantine periods for implantable devices, and in many facilities, for all instruments. These indicators can facilitate improved quality control of sterilization processes by enabling increased frequency of biological monitoring. **HPN**

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Today's rapid readout biological indicators

Circle the one correct answer:

- Biological indicators utilize bacterial spores because spores are difficult to kill and present a significant challenge to the sterilization process.
A. True B. False
- Biological indicators with rapid readout technology rely on a biological signal from germinating and replicating spores.
A. True B. False
- The reference incubation time for a conventional biological indicator is seven days, but rapid readout technology has enabled biological indicators with incubation times of less than 30 minutes.
A. True B. False
- The most effective quality control system for healthcare sterilization uses a combination of physical, chemical, and biological monitoring.
A. True B. False
- Sterilizer printouts from the electronic sensors in the chamber can prove that a sterilization cycle was effective
A. True B. False
- Chemical indicators on the outside of packages are used to test all the sterilization process parameters and prove that the process was effective.
A. True B. False
- Chemical indicators can provide a direct measurement of the lethality of the sterilization process.
A. True B. False
- Biological indicator manufacturers' IFUs are the best reference for where biological indicators and PCDs should be placed in the sterilizer chamber.
A. True B. False
- Rapid readout biological indicators can make it easier to quarantine implantable devices until the BI test is complete.
A. True B. False
- For biological monitoring of steam sterilization, AAMI ST79 recommends weekly, but preferably daily testing, as well as use of biological indicator PCDs with all implant loads.
A. True B. False

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