Living Organisms for Sterility Assurance—

Biological Indicators

Questions to Address:

1. What do AAMI, AORN, and CDC recommend regarding the use of Biological Indicators (BIs)?
2. How does a BI do its job?
3. How does a self-contained BI work?
4. How do Rapid Readout BIs work?
5. What is a D-value?
6. What is SAL and what does it mean to me?
7. How do BIs differ from Chemical Indicators (CIs) in what they can detect?
8. What does 3M recommend regarding use of BIs?

Introduction:

Biological indicators provide direct evidence that the sterilization process conditions are sufficient to kill spores. Having gained the status of a tried and trusted measurement of cycle lethality, BIs are accepted universally by several standards organizations as the “Gold Standard” for monitoring sterilization processes.

Biological indicators have evolved over the past 50 years and continue to provide the only direct measure of sterilization lethality. Results that once took seven days or more now are obtained in one or three hours with self-contained 3M™ Attest™ Rapid Readout Biological Indicators.

The remainder of this tutorial discusses important concepts around BIs. By understanding these concepts, Sterile Processing professionals can make informed decisions regarding the proper use of BIs to monitor their sterilization processes.
Definitions:

- **Biological Indicator (BI):** “Test system containing viable microorganisms providing a defined resistance to a specified sterilization process.”

- **D-value:** “Time or dose required to achieve inactivation of 90% of a population of a test microorganism under stated exposure conditions.”

- **Resistometer:** “Test equipment designed to rapidly produce and precisely control critical parameters associated with a given sterilization process.”

- **Sterile:** “Free from viable microorganisms.”

- **Sterility assurance level (SAL):** “Probability of a single viable microorganism occurring on an item after sterilization.”

- **Sterilization:** “Validated process used to render a product free from viable microorganisms.”

- **Survival-kill window:** “Extent of exposure to a sterilization process under defined conditions where there is a transition from all biological indicators showing growth (survival time) to all biological indicators showing no growth (kill time).”

Frequently Asked Questions:

1. What do AAMI, AORN, and CDC recommend regarding the use of Biological Indicators (BIs)?

   **AAMI:** Association for the Advancement of Medical Instrumentation.  

   • BIs detect conditions that are not able to kill spores.

   • BIs are intended to demonstrate whether the conditions were adequate to achieve sterilization and are the only sterilization process monitoring device that provides a direct measure of the lethality of the process.

   • AAMI recommends routine sterilizer efficacy testing and sterilizer qualification testing with a biological indicator process challenge device (BI PCD). Routine sterilizer efficacy monitoring should be performed at least weekly, but preferably every day that the sterilizer is used. If a sterilizer is designed to be used for multiple types of cycles, then during routine and qualification testing each sterilization cycle type used should be tested. (10.5.3.2) For flash sterilization, each type of tray configuration in routine use should be tested separately. (10.7.4.1) For qualification testing, select one type of tray configuration to test. (10.8.4.1)
• AAMI states the following about loads containing an implantable device: Patient safety could be adversely affected by the implantation of a non-sterile device. The sterilization of implantables should be closely monitored and BIs within PCDs should be used to monitor each load containing implants. The implant should be quarantined until the results of the BI testing are available. (10.5.3.2, 10.6.3)¹

• AAMI recommends the use of BIs for product testing “as part of a complete quality assurance program to ensure the effectiveness of the sterilization process and to avoid wet packs”. (10.9)¹

AORN: Association of periOperative Registered Nurses.

Recommendation XVI
Quality
“XVI.h.2. Steam sterilizers: *Geobacillus stearothermophilus* biological indicators should be used for routine load release, routine sterilizer efficacy monitoring, sterilizer qualification testing, and periodic product quality assurance testing. Routine sterilizer efficacy monitoring should be done weekly, preferably daily, as follows:

• each load containing an implantable device should be monitored with a BI and quarantined until the results of the BI testing are available, and
• one BI PCD should be run in three consecutive empty cycles for sterilizer qualification testing.

If a steam sterilizer is intended to be used for multiple types of cycles (e.g., gravity-displacement, dynamic air-removal, flash), each sterilization mode should be tested.”⁴

CDC: Centers for Disease Control and Prevention

• “If a sterilizer is used frequently (e.g., several loads per day), daily use of biological indicators (BIs) allows earlier discovery of equipment malfunctions or procedural errors and thus minimizes the extent of patient surveillance and product recall needed in the event of a positive BI. Each load should be monitored if it contains implantable objects. If feasible, implantable items should not be used until the results of spore tests are known to be negative.”⁵

• “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.”⁵

• “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.”⁶
2. How does a BI do its job?

Biological indicators detect conditions that are not sufficient to kill spores. Since spores are more resistant than other microbes, they provide a safety margin. Thus, if your process is effective enough to kill a large population of highly resistant spores, it will also kill a lower number of less resistant organisms on the medical devices.

Sterilization cycles are designed to kill spores within the first half of the exposure time. This is often referred to as a half-cycle. In a properly functioning full cycle, the spores should easily be destroyed.

At the beginning of the process all spores are expected to be alive, or survive. By the middle of the process all spores should be killed. Based on this rationale, it is perfectly normal for a BI to be inactivated at half the cycle 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, or superheated steam. This ability to represent lethality of the most resistant form of microbial life is what gives the BI the unique position of “Gold Standard”.

3. How does a self-contained BI work?

A self-contained BI consists of a known 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, hydrogen peroxide gas plasma, and ozone sterilization. *Bacillus atrophaeus* is the most resistant spore for ethylene oxide (EO) and dry heat sterilization.

The self-contained 3M™ Attest™ Biological Indicators consist of a spore strip (spores that are coated on a paper strip) enclosed in a plastic vial along with growth medium contained in a crushable glass ampoule. The cap is designed to allow sterilant to penetrate into the plastic vial, killing the spores and demonstrating that sterilization conditions were met.

After exposure to the sterilization process, BIs must be incubated to determine if any spores survived. Spores start to grow when they come in contact with the nutrients contained in the growth medium and when incubated at the correct temperature.
During this growth process acid metabolites are produced which results in a pH change of the medium, causing the color of the media to turn yellow. If all the spores survive, e.g. the control BI, then the color change may occur within a few hours. However, if only a few spores survive, it typically takes 24-48 hours for a yellow color change to occur. The final incubation time is the time needed to ensure a negative BI.

4. How do Rapid BIs work?

3M Attest rapid readout BIs are similar to conventional self-contained BIs. 3M Attest rapid readout BIs for steam detect the presence of *G. stearothermophilus* by detecting the activity of alpha-glucosidase, an enzyme present within the *G. stearothermophilus* organism. The presence of the enzyme is detected by reading fluorescence produced by the enzymatic breakdown of a non-fluorescent substrate. This creates a fluorescence change, which is detected by the 3M™ Attest™ Auto-reader/Incubator. A fluorescence change indicates a steam sterilization process failure. Non-fluorescence indicates inactivation of the enzyme and an effective sterilization process. 3M Attest rapid readout BIs also indicate the presence of viable *G. stearothermophilus* organisms by a visible color change reaction.

5. What is a D-value?

The D-value is a measure of the resistance of the BI. The D-value is the time required to achieve inactivation of 90% of a population of microorganisms under the stated exposure conditions. D-value is determined in a resistometer that has a small chamber with minimal come-up time and no load.

For example, if a BI containing a population of 1 X 10^6 spores has a D-value of 2 minutes at 121°C (250°F), 90% of the population will be killed within the first 2 minutes of the cycle. During the next 2 minutes, 90% of what was left behind is killed and this phenomenon continues progressively in a logarithmic manner until no survivors can be recovered. (See Figure 2)

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 having a fractional sterilization condition.

For your reference, the Quality Assurance Certificate that accompanies product reports the D-value for each particular lot of 3M Attest BIs. The larger the D-value, the more resistant the microorganism is to destruction.
6. What is SAL and what does it mean to me? \(^7,8\)

Sterility is defined as being free from all living organisms.\(^2\) Since it is not practical to test every device for the absence of microorganisms, the concept of sterility is expressed as a statistical probability. The sterility assurance level, or SAL, defines the probability of a non-sterile unit for a validated sterilization process. Often this is expressed as a probability of \(10^{-6}\), or one chance in one million that a device is not sterile.

The SAL is related to process validation, and cannot be estimated or determined during routine sterilization monitoring. Hospital sterilizer manufacturers and manufacturers of sterile medical devices typically develop and validate steam sterilization cycles using the “half-cycle overkill” method. In this method, the exposure time and conditions required to kill multiple BIs are determined. This cycle time is then doubled to establish the final exposure time. Biological indicators are used to develop and validate processes, and thus estimate SAL, because they have a high population of resistant organisms (spores), providing a uniform and robust challenge to the sterilization process.

Consequently, most steam sterilization processes are based on the half cycle overkill method where the time required to kill the BIs is determined, then doubled to determine the final cycle exposure time. Hence, the final exposure time provides a safety factor.

![Figure 3: Survivor curve representing an SAL of \(10^{-6}\)](image)

7. How do BIs differ from CIs in what they can detect?

A scientific study published in the *American Journal of Infection Control* investigated performance of these monitoring products in suboptimal steam sterilization conditions, e.g. superheated steam and incomplete air removal, and revealed that only BIs are capable of consistently detecting these types of failure modes.\(^9\) Thus, BIs detect common failure modes, whereas CIs may not.

8. What are 3M’s recommended practices for use of BIs?

While national consensus standards recommend weekly, preferably daily, plus loads containing implants, 3M considers Every Load Monitoring with a BI PCD to be a best practice.
Key Learnings:

• BIs provide direct evidence that the sterilization process conditions are sufficient to kill spores. Because of this, BIs are often referred to as the “Gold Standard” in sterility assurance monitoring.

• AAMI ST79 and AORN recommend that BIs be used for routine sterilizer efficacy monitoring and sterilizer qualification testing. Routine sterilizer efficacy monitoring should be done weekly, preferably daily. Loads containing implantables should be monitored with BIs and quarantined until the results of the BI testing are available.

• CDC recommends routine BI monitoring and BI monitoring of all implant loads and that the load be quarantined until the results of the BI testing are available.

• Sterilization cycles are designed to kill spores within the first half of the exposure time. In a properly functioning full cycle, the spores should easily be destroyed. Therefore, it is perfectly normal for a BI to be inactivated in half of the exposure cycle time. As a true measure of lethality, the BI can be depended upon to point out a failed sterilization process if there was a gross or even marginal failure.

• Spores provide a safety margin because they are more resistant than other microbes. If the spores in the BI have been killed then the other microbes on medical devices should have also been killed.

• BIs are typically used by hospital sterilizer and medical device manufacturers for validating sterilization cycles, because of their rigorous and uniform resistance to the process. CIs are not used for validating these types of cycles because they do not provide a direct measure of the lethality of the process.

• Since BIs measure the sterilization process directly, 3M considers running a BI PCD in every load to be the “Gold Standard” for patient safety and improved patient outcomes. Following standards and standardization of practices reduces variability and the chance for errors, and helps ensure that every load of instruments is sterile for every patient.


For more information, call the 3M Help Line: 1-800-228-3957