MesaLabs

Reference Review

Biological Indicators for Sterilization PART 1: History and Use Throughout the Sterilization Life Cycle

BIOLOGICAL INDICATORS FOR STERILIZATION

A Biological Indicator (BI) is a "test system containing viable microorganisms providing a defined resistance to a specified sterilization process".[1] BIs are a type of lethality indicator that utilizes a biological component, typically bacterial endospores, as the sensing element. Other types of lethality indicators include Chemical Indicators (CIs) and physical measurements (equipment instrumentation). Each type has its advantages and disadvantages, which will be discussed in the next section. This article is dedicated to the history of BIs and their use in the Sterilization Life Cycles. Subsequent articles will discuss the controls utilized in their preparation, and the various formats available for the common sterilization modalities.

Lethality Indicators

Lethality indicators are systems that monitor sterilization processes using a well characterized technology. Monitoring the calibrated instrumentation on the sterilizer (to verify the predefined conditions have been met) is a lethality indication activity. CIs utilize ink formulations that undergo a color change upon exposure to predefined sterilization conditions. There are five types of CIs described in detail in the ISO 11140 series. Indicator tape on the outside of a wrapped load is a simple form of a CI generally identified as a process indicator (Type 1). It provides no information about the sterility of the pack's contents, but a color change indicates that the pack has been exposed to the sterilant. Single variable, multivariable, integrating, and emulating indicators (Types 3, 4, 5, and 6, respectively) provide more information about a sterilization process and are designed "to be placed inside individual load items and to assess attainment of the critical process variables at the point of placement".[2] Integrating CIs (Type 5) are designed to mimic the performance of BIs by responding to known variables of a sterilization process. Table 1 provides additional details on CIs as described in ISO 11140–1.

BIs are the gold standard of lethality indicators as they integrate all sterilization parameters involved, both known and unknown. BIs provide direct evidence of sterility assurance as they challenge the sterilizer's ability to kill calibrated strains of highly resistant spore-forming organisms. BIs are often used in conjunction with the other indicators to monitor sterilization processes. Physical measurements and CIs provide real-time or immediate post-processing results, which is their primary advantage over BIs, which require an incubation period in order to achieve the results.

TABLE 1
Description of Chemical Indicator Types and Their Intended Use

Type	ISO 11140-1:2014 definition*	Intended use
Type 1: Process indicators	"Process indicators shall be designed for use with individual items (e.g., packs, containers) to show that the unit has been directly exposed to the sterilization process and to distinguish between processed and unprocessed items."	• Intended to be placed on the outside of individual items. • Shows the item has been exposed to a sterilization process. • Differentiates between processed and unprocessed items. • Example: Indicator tape or indicator labels
Type 2: Indicators for specific tests	"Type 2 indicators are intended for use in specific test procedures as defined in relevant sterilizer/sterilization standards. The requirements for specific test indicators and indicator systems (Type 2 indicators) are provided in ISO 11140-3, ISO 11140-4, and ISO 11140-5."	• Specialty indicator. • Designed for use in specific test procedures outlined in relevant ISO standards. • Example: Bowie–Dick air removal/steam penetration test
Type 3: Single variable indicators	"A single critical process variable indicator shall be designed to react to one of the critical process variables and is intended to indicate exposure to a sterilization process at a stated value (SV) of the chosen critical process variable."	• Intended to be placed inside of items to be sterilized. • Reacts to one critical process variable. • Example: Chemical pellet that melts at a specific temperature
Type 4: Multivariable indicators	"A multicritical process variable indicator shall be designed to react to two or more of the critical process variables and is intended to indicate exposure to a sterilization process at SVs of the chosen critical process variables."	• Intended to be placed inside of items to be sterilized. • Reacts to two or more critical process variables. • Example: Indicator that measures temperature held for a specific amount of time
Type 5: Integrating indicators	"An integrating indicator shall be designed to react to all critical process variables. The SVs are generated to be equivalent to, or exceed, the performance requirements given in the ISO 11138- series for BIs. The minimum SV shall be related to the minimum values required to achieve sterilization as specified in International Standards ISO 11135, ISO 11137 (all parts), ISO 17665 (all parts), or by local regulatory agencies."	• Intended to be placed inside of items to be sterilized. • Reacts to all critical process variables over a specified range of sterilization cycles. • Stated values meet or exceed biological indicator. Performance requirements found in the ISO 11138 series. • Example: Indicator that can be used in a variety of sterilization cycles
Type 6: Emulating indicators	"An emulating indicator shall be designed to react to all critical process variables for specified sterilization processes. The SVs are generated from process variables of sterilization processes as specified in International Standards ISO 11135, ISO 11137 (all parts), ISO 17665 (all parts), or by local regulatory agencies."	• Intended to be placed inside of items to be sterilized. • Reacts to all critical process variables for specific sterilization cycles. • Stated values are generated from the critical variables of the specified sterilization process. • Response does not necessarily correlate to a biological indicator • Example: Indicator that monitors a specific sterilization cycle (e.g., 132°C, 10 minutes)

A BRIEF HISTORY OF BIOLOGICAL INDICATORS

The discovery of microorganisms and their role in spreading disease and infection and causing food spoilage is relatively new. Tyndall, Pasteur, Koch, and Lister were among the early pioneers in developing tools to study and combat problematic organisms.

Koch was perhaps the first person to recognize the value of the spore as a measurement tool. In the early 1880s, he successfully isolated spore-forming bacteria from soil samples and experimented with Bacillus anthracis spores. He recognized that if a sterilization process can inactivate hard-to-kill spores, then it will likely kill other forms of microorganisms. Koch used soil samples (containing an unknown number of spores with an unknown resistance to the process) to evaluate the effectiveness of the sterilization process. When no growth occurred upon culturing the soil sample, the sterilization process was considered adequate.

In the early 1890s, Kilmer began using sealed reference organisms to judge the success of steam sterilization processes used in the manufacture of sterile dressing, "thus pioneering the use of the biological indicator in industrial sterilization".[3] Various methods of food preservation (smoking, salting, drying, etc.) have been in use for thousands of years. The process of canning and heat treating to preserve foods became industrialized in the early 1800s, in part to support the soldiers and sailors in various European military conflicts. *Geobacillus stearothermophilus* proved to be a challenge in producing shelf-stable canned foods and was first characterized by Donk[4] in 1917 after he isolated it from canned corn treated at 118°C for 75 minutes. *G. stearothermophilus* spores are now the most widely used microorganism in BIs for moist heat and some chemical sterilization/ decontamination processes. *G. stearothermophilus* and other spore-forming species are used worldwide as BIs and are recognized by numerous standard-setting organizations.

BACTERIAL ENDOSPORES

Like other bacteria, species of *Bacillus*, *Geobacillus*, and *Clostridium* grow vegetatively; however, when environmental conditions become unfavorable, organisms from these genera have the ability to sporulate leading to the formation of dormant spores. This process is a survival mechanism rather than a reproductive function. When the environment remains favorable, the organisms will continue to grow and reproduce in the vegetative state.

Spores are one of the most stable forms of life known and can remain viable for millions of years in this "dormant" state (Figure 1). Although the spore is considered dormant, it does contain active enzymes that trigger the spore to germinate in a short period of time when environmental conditions once again become favorable. In the vegetative state, the cells are susceptible to environmental stresses; however, while in the spore state, the organism gains significant resistance to these stresses.

In short, the characteristics that make bacterial spores ideal for use in BIs include:

1. They can withstand environmental stresses, including sterilization conditions, well beyond that of most other microorganisms.
2. They integrate known and unknown process parameters of a sterilization process.
3. They have a gradual, largely predictable inactivation phase (kill curve/death curve).
4. They can be "calibrated" (manufactured to contain a known spore count with a defined resistance to a sterilization process).

5. They have extreme longevity, which is desirable for maintaining a long shelf-life.
6. They can be grown in sufficient quantities to produce large batches of uniform indicators.
7. They allow results to be binary (a BI positive for growth indicates non-sterility; a BI showing no-growth indicates sterility).

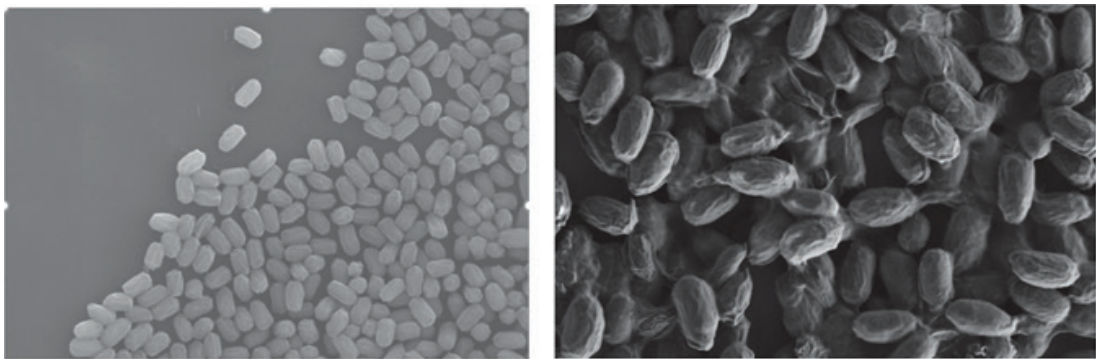

FIGURE 1 Micrograph of spores of *B. subtilis* (5k magnification) and *G. stearothermophilus* (7.5k magnification).

BI USES THROUGHOUT THE STERILIZATION LIFE CYCLE

A sterilization life cycle generally consists of three elements: cycle development, cycle validation, and routine monitoring. BIs and/or inoculated product are used in each of these three elements.

Cycle Development

Developing a proper sterilization cycle is key to producing sterile and functional product. Increasingly, pharmaceutical and medical device products are becoming heat labile, and moist heat sterilization by the overkill method is not an option in these cases. **It is important to understand the nature of the bioburden (total quantity, identity, resistance, presence/absence of spore formers) as these are the true target of the sterilization process.** It is equally important to know how the product influences the resistance of the organisms. Product components (active ingredient, excipients, preservative, etc.) may protect the organisms by coating them or causing them to clump, increasing their resistance to the sterilization process, or the product components may sensitize the spores, making the organism more vulnerable to the sterilization process.[5]

Performing D-value studies on inoculated product provides this information. A common practice in these studies is to use *G. stearothermophilus* spores (or other recognized organisms referenced in the standards) as a substitute for the bio-burden. This is a conservative approach, as these spores are typically substantially more resistant than the bioburden organisms and by design, significantly outnumber the bioburden organisms.

D-value studies are not restricted to product terminal sterilization processes but should also be performed on enclosure components used in aseptic fill. Studies performed on stoppers have shown a wide range of D-values depending on their composition and coatings.[5]

D-value studies are key to proper cycle development, and regulatory bodies expect to see these data to support the design of the final process. These studies can be performed by the firm, but commonly they are provided as a service by BI manufacturers. BI manufacturers not only supply spore suspensions, but they also possess resistometers[6] (test equipment required to perform D-value assessments).

Cycle Validation
The purpose of validating a sterilization cycle is to ensure that the process will consistently render the load sterile. The role of the BI in this process is to demonstrate that sterilization conditions are met by monitoring areas in the load that have been identified as having lower delivered lethality. In the case of thermal sterilization processes, these areas are often referred to as "cold spots" or "slow-to-heat areas" and are identified through thermal mapping of the load. In the case of chemical sterilization, CIs may be a useful tool in identifying the lower areas of lethality, which can then be monitored by BIs during the actual cycle validation. The practice of mapping with CIs and then monitoring with BIs is commonly used when validating isolators and associated equipment used in aseptic filling operations.

Routine Monitoring
The purpose of using BIs for routine monitoring of sterilization processes is to ensure that the system has not drifted outside of the validated state. The monitoring frequency will depend on the firm's practices and regulatory requirements. These can range from monitoring each cycle to daily or weekly monitoring.

BIs should be placed in the most difficult to sterilize (worst- case) location in the load and/or chamber, which should have been identified in the process development and validation studies. Retrieving BIs embedded in sterilizer loads can be problematic, as the packaging and/or load will become compromised. An alternative to the embedding of BIs in product is the use of Process Challenge Devices (PCDs).

Learn more about BIs and PCDs in the next part of the chapter release.

REFERENCES

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