⟨1229.5⟩ BIOLOGICAL INDICATORS FOR STERILIZATION

INTRODUCTION

A biological indicator (BI) is a well-characterized preparation of a specific microorganism that has known resistance to a specific sterilization process.[1] BIs are used to demonstrate the effectiveness of processes that render a product sterile in its final package or container, as well as the effectiveness of the sterilization of equipment, product contact materials, and packaging components as required. BIs may also be used to monitor established sterilization cycles and are used for periodic reassessment of sterilization process effectiveness. BIs are process aids and can support the correlation of physical parameters to microbiological destruction. Microorganisms recognized as suitable for BIs are spore-forming bacteria, because the spores of these microorganisms are significantly more resistant than the vegetative cells that comprise the majority of bioburden in or on materials.

PROPER USE OF BIOLOGICAL INDICATORS

BIs provide microbiological evidence of process effectiveness that should be correlated to physical measurements (see *Sterilization of Compendial Articles* ⟨1229⟩). Microbiological resistance to sterilization varies with the physical conditions; however, there are no established means for accurately predicting microbial destruction based solely on physical measurements, with the exception of radiation sterilization. BI placement locations within or on materials to be sterilized are chosen to confirm that the desired sterilizing conditions have been attained.

BIs are typically spore-forming bacteria of the genera *Geobacillus*, *Bacillus*, and *Clostridium*. Preference in BI selection should be given to well-characterized strains.

RESPONSIBILITIES

BI Manufacturer's Responsibility

The responsibility for determining the performance characteristics of each BI lot resides with the BI manufacturer. The manufacturer should provide, with each lot of BIs, a certificate of analysis that attests to the validity of BI performance claims. The manufacturer should provide information concerning the microbial population and resistance (*D* and *z* values, respectively, where appropriate) as well as storage and expiry information. The BI manufacturer may choose to include survival or kill times for the BI in their documentation. The resistance of the BI should be determined by the manufacturer under defined conditions. The manufacturer should provide directions for use, including the medium and conditions used for the recovery of microorganisms after exposure to the sterilization process. Disposal instructions also should be provided by the manufacturer of the BI.

BI User's Responsibility

When BIs are purchased, their suitability for use in a specific sterilization process must be established. The BI user should obtain a certificate of analysis for each lot of BIs and verify the manufacturer's label claims for spore population (see *Biological Indicators—Resistance Performance Tests* ⟨55⟩). When a BI is used in accordance with the BI manufacturer's directions, the resistance of the BI need not be reconfirmed.

User-Prepared Biological Indicators

A user of BIs may elect to propagate spore crops of a single species for use as a suspension. Alternatively, these spore suspensions may be purchased from a BI manufacturer. When liquid suspensions are applied to a substrate, it is the user's responsibility to determine the population and resistance of the microorganism used. The resistance determined for liquid suspensions relates only to other lots of the same suspension and is not representative of how that microorganism will perform on a substrate or in a different suspending medium. In these circumstances, the BI resistance and population should be re-established (see ⟨55⟩).

CHARACTERIZATION OF BIOLOGICAL INDICATORS

The use of BIs should include procedures for their acceptance and control. The following elements outline the major considerations. Resistance performance is addressed separately in ⟨55⟩, which provides methods for evaluating BI resistance.

Packaging and Storage

Store under the conditions recommended on the label or under validated conditions, and protect from light, toxic substances, excessive heat, and moisture.

[1] Microbial retention challenges as described in *Sterilizing Filtration of Liquids* ⟨1229.4⟩ are not BIs.

Expiration Date

Use within the BI's labeled or determined expiration date.

Identification

Where identification of the BI species is deemed necessary, as in the course of an investigation into unusual results, use either a phenotypic or genotypic identification method (see *Microbial Characterization, Identification, and Strain Typing* ⟨1113⟩ for additional information).

Purity

By examination of the colonies derived from the spores on a suitable plate culture medium, determine that there is no evidence of contamination with other microorganisms.

Disposal

Prior to discarding used spores, sterilize using a method recommended by the BI manufacturer or other equivalent means.

TYPES OF BIOLOGICAL INDICATORS

A BI is a well-characterized preparation of a specific bacterial spore of known resistance to a specific sterilization process. Some BIs may contain two different species and concentrations of bacterial spores for use in the evaluation of two different sterilization processes.

One form of BI preparation includes spores that are placed on a carrier (e.g., a disk or strip of paper, glass, plastic, metal, or other material) and may be packaged to maintain the integrity and viability of the spores inoculated onto the carrier. The carrier and primary packaging should not be damaged or degraded by the specific sterilization process. Another preparation of BIs is a spore suspension that is inoculated on or into representative units of the article to be sterilized. A surrogate article may be used if it is not practical to inoculate the actual article. A surrogate article is a preparation that differs in one or more ways from the actual article but performs as the actual article during cycle development, validation, and routine use. The physical design of actual or surrogate articles can affect the resistance of spore suspensions that are inoculated on or into an article (see ⟨55⟩). In the case of liquid inoculated products, it is essential to determine the population, *D* value (and, in terminal sterilization applications, *z* value) of the relevant BI spore in the liquid product, and any simulated product substrate (if utilized).

A third form of BI is a sealed system that includes the growth medium (either in direct contact with the BI during the sterilization or placed in contact with the BI after sterilization) for recovery of process-exposed BI microorganisms. Some BI systems may contain a growth indicator or sensor in addition to growth media.

SELECTION FOR SPECIFIC STERILIZATION PROCESSES

The selection of a BI requires knowledge of the resistance of the BI system to the specific sterilization process. It must be established that the BI system provides a challenge to the sterilization process greater than the resistance of the native bioburden. The recommendations for BI with each sterilization process are not exclusive; they represent only the more common choices.

Steam Sterilization by Direct Contact

For steam sterilization by direct contact, the commonly used BI contains spores of *G. stearothermophilus* (ATCC 12980 or ATCC 7953), a thermophilic microorganism with a moist heat resistance substantially greater than that of most vegetative microorganisms (see *Steam Sterilization by Direct Contact* ⟨1229.1⟩).

Moist Heat Sterilization of Aqueous Liquids

Heat-resistant spore-forming microorganisms such as *C. sporogenes* (ATCC 7955), *B. subtilis* (ATCC 35021), or *B. atrophaeus* (ATCC 9372) are used. *B. subtilis*, *B. atrophaeus*, and *C. sporogenes* are preferred for use in sterilization of aqueous solutions or where their lower thermal resistance is more appropriate.

Dry Heat Sterilization

For dry heat sterilization, spores of *B. atrophaeus* (ATCC 9372) are typically used (see *Dry Heat Sterilization* ⟨1229.8⟩). Where dry heat depyrogenation has been demonstrated (*Dry Heat Depyrogenation* ⟨1228.1⟩), sterilization by dry heat need not be confirmed, and a BI is not required. The elevated temperatures required to depyrogenate materials are more than sufficient to sterilize the materials at the same time.

Ionizing Radiation

The use of a resistant BI is unnecessary for the evaluation of radiation sterilization processes. Dose setting involves the evaluation of preirradiation bioburden as well as dosimetric evaluation and allied tests as defined in ISO 11137-1, -2, and -3, as well as in *Radiation Sterilization* ⟨1229.10⟩.

Gas Sterilization

For ethylene oxide sterilization, spores of *B. atrophaeus* are commonly used. For other gaseous agents, spores of *G. stearothermophilus* or *B. atrophaeus* are commonly used (see *Gaseous Sterilization* ⟨1229.7⟩).

Chemical Sterilization

The sterilization of items using a liquid sterilant is accomplished using spores of an appropriate strain such as *B. atrophaeus*, *B. subtilis*, or other appropriate spore-forming species, as determined by the user. Whichever strain is chosen for this purpose should have greater resistance than does the bioburden.

Vapor Phase Sterilization

The biphasic nature of these materials precludes the accurate determination of specific lethal conditions (for establishment of *D* values, see *Vapor Phase Sterilization* ⟨1229.11⟩). BIs using either *G. stearothermophilus* or *B. atrophaeus* have been utilized in the evaluation of these processes.

REFERENCES

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