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Performance Requirements for Biological Indicators: Understanding BI Resistance

LEIVA, MPH, PHD, SUBJECT MATTER EXPERT IN MEDICAL DEVICE REPROCESSING

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LEARNING OBJECTIVES

1. Understand the key parameters that determine biological indicator resistance
2. Explain the testing methods used to determine biological indicator resistance
3. Identify the most suitable practices for using biological indicators in healthcare facilities

Sterilization is a unique process that cannot be confirmed by inspection or testing alone. In fact, if one tries to demonstrate that a package or container is sterile by opening it to check, the sterility is lost, and the item must be reprocessed. Therefore, Sterile Processing (SP) professionals rely on a holistic approach that includes equipment monitoring (e.g., pressure, time, temperature) and verification (i.e., Bowie-Dick test). Pack control (e.g., internal and external indicators) and load control are also implemented, which is where BIs are used.

BIs are essential tools for sterilization validation and monitoring because they directly challenge the sterilization process with highly resistant microorganisms. Their value depends on a well-defined and reproducible level of resistance that reflects worst-case conditions. To properly select, interpret and rely on BIs, SP professionals must understand the key parameters that determine BI resistance.

This lesson identifies the key parameters that determine BI resistance and the testing methods used to assess it, and explains the appropriate use of BIs in the healthcare setting.

Objective 1: Understand the key parameters that determine biological indicator resistance

The key parameters that determine BI resistance (see **Table 1**) are scientifically defined and standardized to ensure that the indicators provide meaningful assurance of sterilization effectiveness.

The foundation of BI resistance begins with the type of microorganism used. BIs contain bacterial spores selected for their known resistance to specific sterilization modalities. For example, *Geobacillus stearothermophilus* is commonly used for steam and hydrogen peroxide sterilization, while *Bacillus atrophaeus* is used for ethylene oxide (EO). The selected organism must exhibit greater resistance to the sterilization process than the typical

Parameter	Description	Why It Determines BI Resistance	Key Standards / Examples
Microorganism Type	Specific bacterial spores selected for each sterilization modality	Different microorganisms have different resistance mechanisms. Selecting the most resistant organism for a given process ensures a worst-case challenge.	*Geobacillus stearothermophilus* → Steam, H_2O_2 *Bacillus atrophaeus* → Ethylene oxide (EO)
Spore Population (CFU)	Number of viable spores on the BI carrier	Higher initial populations require greater lethality to achieve sterility, increasing the challenge to the sterilization process.	≥ 100,000 CFU required by ANSI/AAMI/ISO 11138-1
D-Value	Time (or dose) required to achieve a 90% (1-log) reduction under defined conditions	Quantifies resistance; higher D-values mean greater resistance and a more stringent test of sterilization effectiveness.	Determined per BI lot using BIER vessels
Exposure Conditions	Specific temperature, pressure, time and sterilant concentration used to define resistance	Resistance is only meaningful when measured under standardized, controlled conditions.	Example: D-value at 121°C for steam
Environmental & Storage Conditions	Temperature, humidity and shelf life during storage and handling	Poor storage can reduce spore viability or alter resistance, invalidating BI results.	Manufacturer storage specifications

Table 1: Key Parameters That Determine BI Resistance

product bioburden. This ensures that inactivation of the BI implies inactivation of naturally occurring microorganisms. Resistance is not universal; a microorganism that is highly resistant to moist heat may not be equally resistant to chemical sterilants; therefore, correct organism selection is a primary determinant of BI resistance.

Another critical parameter is the spore population, or the number of viable spores present on the BI. ANSI/AAMI/ISO 11138-1:2017(R)2024, developed by the American National Standards Institute (ANSI), the Association for the Advancement of Medical Instrumentation (AAMI), and the International Organization for Standardization (ISO), requires a population of at least 100,000 colony-forming units (CFU)1; however, some manufacturers may provide higher populations.

The decimal reduction time (D-value) is the most widely used quantitative measure of BI resistance. It represents the time required under defined conditions to reduce the spore population by 90% (one logarithmic reduction). For example, a D-value of 1.5 minutes at 121°C means it takes 1.5 minutes at that temperature to reduce the population by one log. In other words, after 1.5 minutes, the population decreases from 100,000 to 10,000. After another 1.5 minutes, it drops from 10,000 to 1,000, and so on, until no viable spores remain. D-values are specific to each BI and manufacturing lot and are determined through rigorous testing in BI evaluation resistometer (BIER) vessels.

The carrier material and method used to apply spores also influence BI resistance. Spores may be applied to paper strips or loaded onto a self-contained system. Uniform inoculation and secure adhesion of spores to the carrier are essential to ensure consistent resistance. Poor carrier design or inconsistent spore application can artificially increase or decrease resistance, compromising the reliability of the BI. Environmental conditions, such as temperature, humidity and shelf life, can influence BI resistance over time. Manufacturers specify storage conditions to preserve resistance characteristics, and deviations can degrade spore viability or alter resistance. This makes proper handling and storage essential for maintaining BI integrity.

AAMI= Association for the Advancement of Medical Instrumentation; ANSI= American National Standards Institute; BI= biological indicator; BIER= biological indicator evaluation resistometer; CAPA= corrective and preventive action; CFU= colony forming units; ISO= International Organization for Standardization

Objective 2: Explain the testing methods used to determine the resistance of biological indicators

To ensure consistency and reliability, internationally recognized standards, primarily ANSI/AAMI/ISO 11138 part 1, and ANSI/AAMI/ISO 14161:2009, define testing methods for establishing and applying BI resistance.[2,3] The U.S. Food and Drug Administration (FDA) sets regulatory expectations for how these methods are justified, documented and used in regulated manufacturing.[4,5,6] Understanding these testing methods is essential for quality,

Characteristic	ANSI/AAMI/ISO 11138	ISO 14161	FDA
Defines resistance test methods	✔	✘	✘
Determines D- and z-values	✔	✘	✘
Guides BI selection for processes	✘	✔	✔
Requires correlation with process data	✘	✔	✔
Evaluates documentation & rationale	✘	✘	✔
Enforces CAPA & trending	✘	✘	✔

Table 2: Key Requirements for BIs Defined by Regulatory Standards

AAMI= Association for the Advancement of Medical Instrumentation; ANSI= American National Standards Institute; BI= biological indicator; CAPA= corrective and preventive action; FDA= U.S. Food and Drug Administration; ISO= International Organization for Standardization

regulatory and sterile processing professionals (see **Table 2**).

ISO 11138 is the primary standard governing the manufacture and characterization of BIs.[2] Its testing methods are designed to establish measurable resistance characteristics under controlled and reproducible conditions. A core testing method defined in ISO 11138 is the determination of the D-value. D-value testing involves exposing a series of inoculated BI carriers to a defined sterilization process for increasing exposure times or doses. After exposure, the indicators are incubated under specified growth conditions, and the number of surviving spores is assessed. This method provides a quantitative measure of BI resistance and enables comparisons across different indicators and processes.

ISO 11138 also includes evaluation of the Z-value, which describes how temperature changes affect microbial resistance. Z-value testing involves determining D-values at multiple temperatures and calculating the temperature change needed to alter the D-value by one log. This testing supports the understanding of resistance behavior across a range of operating conditions and ensures that BI performance remains predictable. Additionally, ISO 11138 recommends the testing of spore population consistency, carrier integrity, recovery efficiency, and growth promotion. Resistance testing must demonstrate repeatability and stability throughout the BI's shelf life. These methods ensure that the resistance characteristics declared by the manufacturer are scientifically valid and reproducible.

While ISO 11138 focuses on how resistance is established, ISO 14161 addresses how resistance data are used and interpreted in sterilization process validation and routine monitoring.[3] ISO 14161 does not introduce new resistance test methods; instead, it provides guidance on applying resistance data generated under ISO 11138. ISO 14161 emphasizes that resistance testing must reflect worst-case conditions relevant to the actual sterilization process. It recommends using BIs with resistance characteristics suitable for the sterilization modality and load configuration. The standard also highlights the importance of correlating BI results with physical and chemical monitoring data. Resistance testing supports validation, but ISO 14161 cautions that BI performance alone does not define process effectiveness. Instead, resistance data must be interpreted within a validated process framework that includes temperature, pressure, concentration, and exposure time.

The FDA does not publish a single resistance testing standard equivalent to ISO 11138. Still, it recognizes consensus standards and evaluates BI resistance testing within the broader context of process validation and quality systems. From an FDA perspective, resistance testing must be scientifically justified, documented and appropriate for the intended use. The FDA expects manufacturers to demonstrate that BIs used in sterilization validation have well-characterized resistance, typically supported by ISO 11138-compliant testing; however, FDA requirements go further by emphasizing process relevance. Resistance testing data must be applicable to the specific product, packaging, load configuration, and sterilization parameters. Relying solely on supplier certificates is insufficient without a documented rationale. During inspections and submissions, the FDA evaluates how resistance testing supports installation, operational and performance qualification, commonly known as IQ/OQ/PQ. The FDA also expects manufacturers to trend BI performance over time and to investigate any unexpected changes in resistance or failures under formal corrective and preventive action (CAPA) systems.

ISO 11138 defines how resistance is measured, and ISO 14161 explains how resistance data should be applied. The FDA ensures regulatory accountability for how resistance testing supports patient safety. ISO standards provide the technical foundation. In contrast, FDA oversight ensures that resistance testing is not treated as an isolated laboratory exercise but as part of a controlled, validated sterilization process.

Guidance	Routine Monitoring	Implant Loads	Sterilization Failures	Process Validation
FDA – Process Validation (2020)	✔ Ongoing process monitoring	✔ Validation of implant processes	✔ Investigation of deviations	✔ IQ/OQ/PQ framework
FDA – 21 CFR Part 820	✔ Documentation & records	✔ Risk-based controls	✔ CAPA requirements	✔ Validation required
FDA – BI 510(k) Guidance (2021)	✔ Use of cleared BIs	✔ Appropriate BI selection	✔ Reliable BI performance	✔ Confirms BI suitability
ANSI/AAMI ST79	✔ Weekly BI testing	✔ Quarantine until BI negative	✔ Sterilizer removal from service	✔ Supports validated processes
ANSI/AAMI/ISO 11138	✔ Resistance characteristics	✔ High-resistance BI design	✔ Reliable failure detection	✔ Defines BI performance
ANSI/AAMI/ISO 14161	✔ BI placement & handling	✔ Conservative release guidance	✔ Interpretation of positive BI	✔ BI use in validated processes

Table 3: Integration of BIs into Regulated Sterilization Processes

AAMI= Association for the Advancement of Medical Instrumentation; ANSI= American National Standards Institute; BI= biological indicator; CAPA= corrective and preventive action; FDA= U.S. Food and Drug Administration; IQ/OQ/PQ= installation

Objective 3: Identify the most suitable practices for using biological indicators in healthcare facilities

BIs are a cornerstone of sterility assurance in healthcare facilities. Unlike physical and chemical monitors, BIs directly challenge the sterilization process with highly resistant microorganisms, providing the strongest evidence that sterilization conditions were effective. Still, their value depends on their correct, consistent use and alignment with clinical risk.

In healthcare facilities, BIs are used to verify sterilizer performance, not to test individual instruments. Their primary role is to confirm that a sterilization process can consistently achieve microbial inactivation under worst-case conditions. Because sterility cannot be proven by inspection alone, BIs provide critical assurance that validated parameters—time, temperature, pressure, or sterilant concentration—are achieving their intended effect. BIs should always be part of a multi-layered monitoring system that includes mechanical monitoring (cycle parameters) and chemical indicators. Using BIs as a standalone control is inappropriate and can lead to false confidence or delayed detection of process failures.

Self-contained BIs are often preferred in clinical settings because they reduce handling errors, simplify incubation, and provide faster, more reliable results. Healthcare facilities should use BIs that are FDA cleared, include clear instructions for use, are compatible with existing sterilization equipment and cycles, and are included in standardized process challenge devices (PCDs).

Determining how often to use BIs is a critical aspect of suitability. Best practices in healthcare, such as ANSI/AAMI ST79:2017/(R)2022, recommend routine BI testing of each sterilizer at least weekly, with more frequent testing for high-risk applications, such as the release of implantable devices.[7] Best practice dictates that implants be quarantined until BI results are confirmed negative. If emergency release is unavoidable, facilities should perform a documented risk assessment and notify clinical leadership. This approach balances patient safety with clinical urgency while maintaining accountability and traceability; however, it introduces an ethical dilemma regarding the standard of care, as it takes a process-based rather than a risk-factor approach. For instance, a young and healthy athlete in need of an anterior cruciate ligament (ACL) repair will require interference screws, the implant device, for which the loads involved in that surgery will require release only after a BI confirms sterility. On the other hand, an elderly patient with multiple risk factors undergoing a laparoscopic procedure may not benefit from loads released with a negative BI. This example illustrates the rationale for a process-based approach rather than a risk-factor approach. BIs should also be used after installation or relocation of a sterilizer, following major repairs or maintenance, and after process changes or sterilization failures.

Proper BI placement is essential. BIs should be placed in the most challenging location for sterilant penetration, often within a PCD. This location represents worst-case conditions and provides confidence that the entire load has been adequately sterilized if the BI shows no growth. For routine loads, placing the BI in a representative load configuration is critical. Random or inconsistent

placement reduces the value of BI testing and may fail to detect process weaknesses. The most suitable BI use also depends on correct handling and interpretation. BIs must be stored according to the manufacturer's instructions and incubated at the specified temperature and duration. Deviations can lead to false results and undermine sterility assurance.

Interpreting BI results requires context. A negative BI result should be reviewed alongside mechanical data and CI results. Conversely, a positive BI result must be treated as a serious event, triggering immediate investigation, removal of the sterilizer from service, and evaluation of all affected loads.

The most effective use of BIs occurs when they are fully integrated into the facility's quality management and infection prevention programs (see **Table 3**). Written procedures, staff training, competency assessments, and documentation of results ensure consistent application and continuous improvement. Trending BI results over time can help identify early signs of equipment degradation or process drift, allowing corrective action before patient safety is compromised.

Conclusion

A single attribute does not define BI resistance; rather, it is a combination of scientifically controlled parameters. Microorganism selection, spore population, D-value, Z-value and environmental stability all contribute to a BI's ability to provide a meaningful challenge to sterilization processes. Understanding these parameters enables SP professionals to select appropriate BIs, interpret results correctly, and maintain confidence in sterilization monitoring.

Testing methods used to determine BI resistance rely on scientifically defined procedures centered on

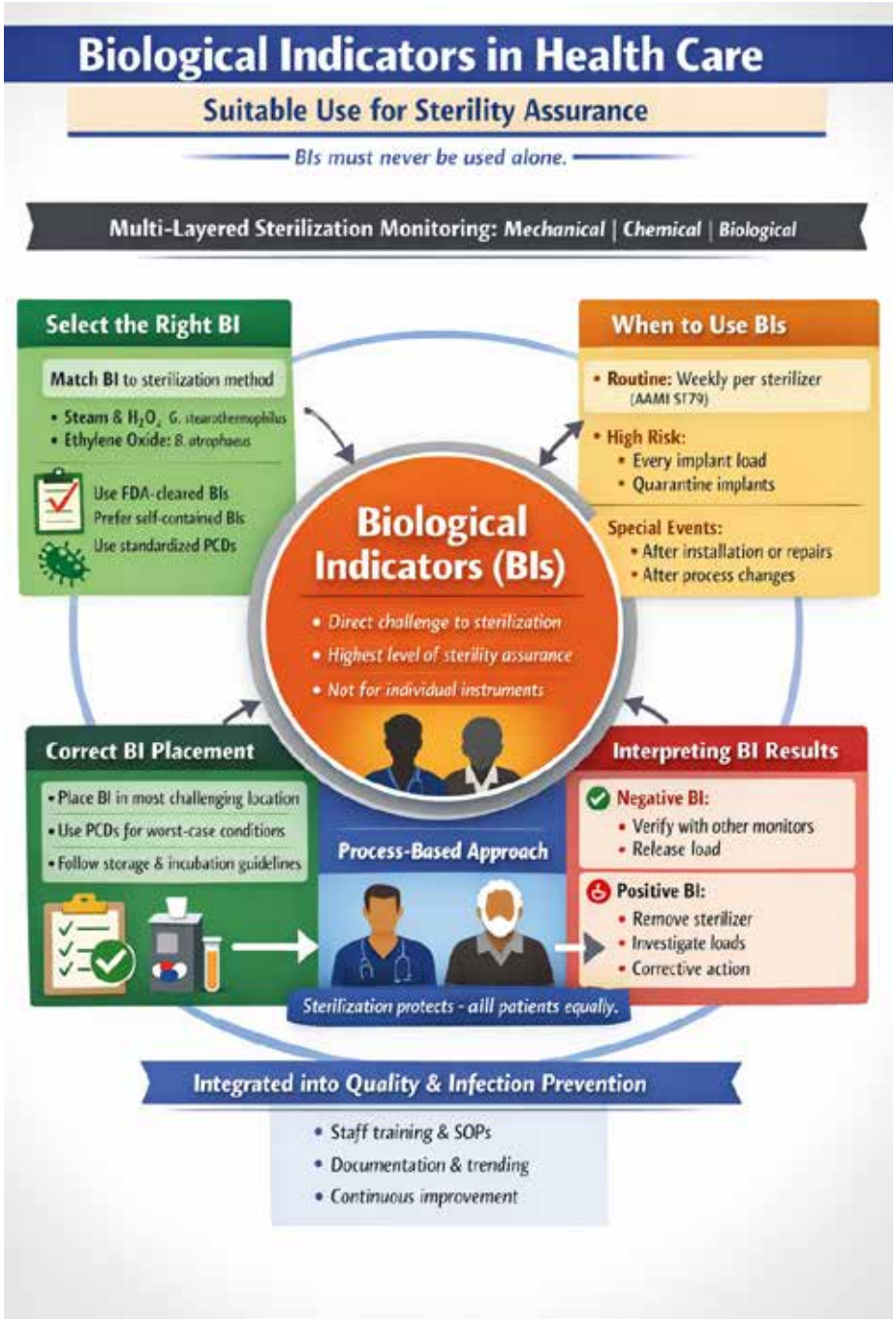

Figure 1: Best Practices for Biological Indicator Use in Healthcare

This is an AI-generated figure (ChatGPT). The authors reviewed and verified the content's accuracy and appropriateness.

D-value, Z-value and population consistency, as described in ISO 11138, while ISO 14161 guides the proper application and interpretation of this resistance data during validation and routine monitoring. From a regulatory perspective, the FDA outlines requirements through robust justification, documentation and integration to a regulated quality system. Identifying the most suitable way to use BIs in healthcare facilities requires more than routine testing; it involves selecting the right BI, using it at appropriate frequencies and interpreting results within a comprehensive monitoring system. When properly applied, BIs provide robust assurance that sterilization processes are functioning as intended, supporting safe patient care and regulatory compliance. P

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7. Association for the Advancement of Medical Instrumentation. *ANSI/AAMI ST79:2017/(R)2022 Comprehensive guide to steam sterilization and sterility assurance in health care facilities.*

CRCST Self-Study Lesson Plan Quiz: Performance Requirements for Biological Indicators: Understanding BI Resistance

Lesson No. CRCST 207 (Technical Continuing Education – TCE) · Lesson expires April 2029

1. Sterilization is considered a special process because:
   a. It only applies to implantable medical devices
   b. Sterility can be verified by visual inspection
   c. Sterility cannot be fully verified without compromising the product sterility
   d. It eliminates all infection-causing microorganisms

2. Which monitoring approach best reflects a holistic sterility assurance system?
   a. Use of process challenge devices (PCDs)
   b. Use of BIs and chemical indicators (CIs)
   c. Visual inspection of packaging
   d. Use of equipment monitoring, verification tests, pack control and BIs

3. What is the primary purpose of a BI?
   a. To test individual instruments for sterility
   b. To replace CIs
   c. To directly challenge the sterilization process with resistant microorganisms
   d. To measure packaging integrity

4. Which microorganism is most used in BIs for steam sterilization?
   a. *Escherichia coli*
   b. *Staphylococcus aureus*
   c. *Clostridium difficile*
   d. *Geobacillus stearothermophilus*

5. According to ANSI/AAMI/ISO 11138-1, what is the minimum required spore population for a BI?
   a. 1,000 CFU or 103
   b. 10,000 CFU or 104
   c. 50,000 CFU or 102.5
   d. 100,000 CFU or 105

6. What does the D-value of a BI represent?
   a. The total time required to sterilize a load
   b. The temperature needed to destroy all spores
   c. The time or dose required to reduce the spore population by 90%
   d. The incubation time for BI results

7. In which equipment are D-values for BIs determined?
   a. Steam sterilizers used in hospitals
   b. Incubators used for routine monitoring
   c. Bowie-Dick test devices
   d. Biological Indicator Evaluation Resistometer (BIER) vessels

8. Which ISO standard focuses on how BI resistance is established?
   a. ANSI/AAMI ST79
   b. ANSI/AAMI/ISO 14161
   c. ANSI/AAMI/ISO 11138
   d. ISO 9001

9. According to best practices identified in ANSI/AAMI ST79, how often should BIs be used for loads containing implantable devices?
   a. In every load containing implants
   b. Weekly
   c. Monthly
   d. Only after sterilizer repairs

10. Which action is most appropriate following a positive BI result?
   a. Release the load if the CIs passed
   b. Repeat the BI test without investigation
   c. Remove the sterilizer from service, initiate an investigation and reprocess the load
   d. Repeat the BI three times to verify the results

11. A negative BI result should be reviewed alongside mechanical data and CI results.
   a. True
   b. False

12. BIs should be placed:
   a. In a moderately challenging location for sterilant penetration
   b. In the most challenging location for sterilant penetration, often within a PCD
   c. In a corner directly opposite the CI
   d. In at least three locations to verify sterilant penetration

13. For routine loads, placing the BI in a representative load configuration:
   a. Is optional
   b. Is critical because inconsistent placement may fail to detect process weaknesses
   c. Is a regulatory requirement identified in ANSI/AAMI ST79
   d. Is a step best performed by the most experienced SP technician

14. Implants should be quarantined until BI results are confirmed negative. If emergency release is unavoidable:
   a. The patient should be informed before the procedure
   b. The surgeon must sign off on its use, and the risk manager must document the incident
   c. A risk assessment should be performed, and clinical leadership should be notified
   d. All the above

15. BI performance alone defines process effectiveness.
   a. True
   b. False

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