Evaluating Biological Indicator Performance in Saturated Steam Sterilization for Central Sterile Supply Department Monitoring

BY EMERSON APARECIDO MIGUEL

Saturated steam sterilization is the most widely used method in Sterile Processing Departments/Central Sterile Supply Departments (SPDs/CSSDs). Its widespread adoption is due to its high level of safety, efficacy, and favorable cost-benefit ratio compared to other technologies.

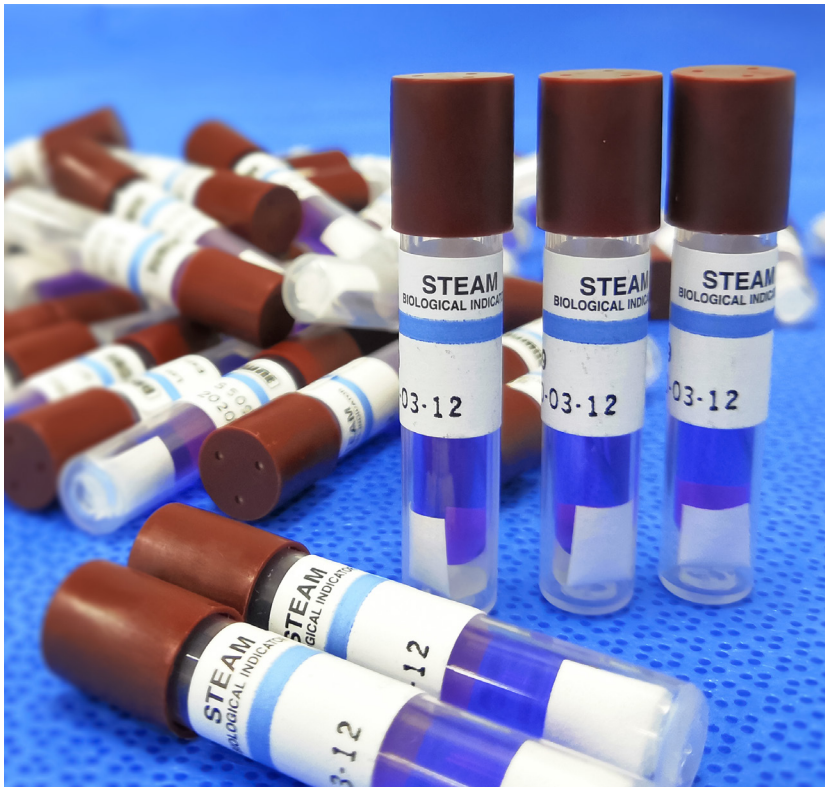
To ensure process reliability, several critical steps must be rigorously controlled. These include cycle validation, preventive and corrective maintenance programs, periodic calibration of sterilizer instruments, and the structuring of routine monitoring.

The daily control of this process relies on physical, chemical, and biological indicators, which are essential for the safe operation of the CSSD. Among these, the biological indicator (BI) is widely recognized as the most sensitive and reliable tool for demonstrating microbial kill on medical devices. This product is internationally standardized and regulated by industrial manufacturing guidelines, such as International Organization for Standardization (ISO) 11138-1 and ISO 11138-3.[1,2]

Although the use of BIs is indispensable in the monitoring strategy, a crucial question arises: do all

Learning Objectives

1. **Discuss how biological indicator (BI) performance can be evaluated during routine steam sterilization cycles.**
2. **Identify practical considerations for assessing BIs based on sterilizer characteristics and monitoring practices.**
3. **Compare rapid incubation results with pH-based growth detection and their impact on interpreting BI results in daily use.**



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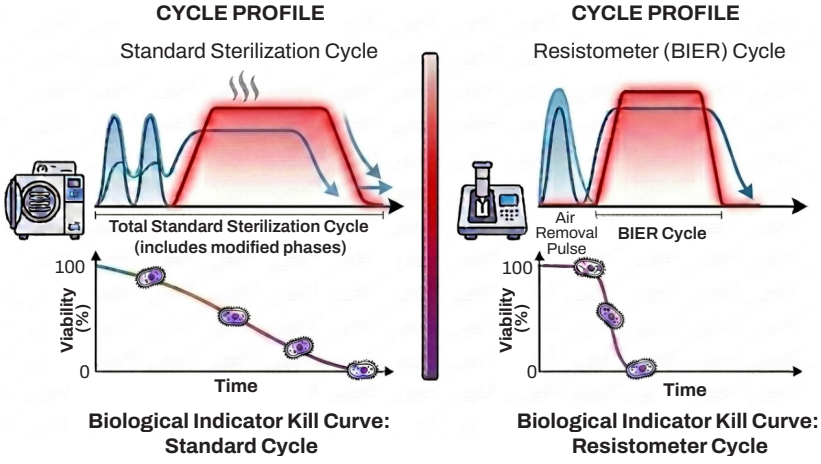

Figure 1. Differences between standard CSSD sterilization cycles and BIER resistometer cycles used by BI manufacturers

products available on the market offer the same quality? Furthermore, is it possible to evaluate the actual performance of the chosen BI within the CSSD itself?

This article examines these questions, aiming to clarify fundamental points to guide end-users in making informed choices among the BI products available for CSSD monitoring.

LEARNING OBJECTIVE 1:

Discuss how BI performance can be evaluated during routine steam sterilization cycles.

The routine use of BIs or their application in process validation stages does not aim to evaluate the performance of the indicator itself, but rather the performance of the sterilization equipment. During process validation, specifically in the performance qualification (PQ) phase, BIs are used to demonstrate the success of bioburden inactivation from a biological perspective. At this stage, no tests are performed to determine the quality of the BI used as a challenge. Product selection is generally based on documents provided by the manufacturer attesting to compliance with regulatory requirements. Consequently, if the indicator is out of specification, there is a risk of hidden failures occurring during the release of falsely sterilized loads.

Given this, how can the quality of the sterilization monitor be evaluated? Taking into account the technology of current steam sterilizers, it would be feasible to configure dedicated program cycles to simulate an insufficient exposure relative to the kill time of the tested lot population. In this way, the expected result after incubation would be bacterial growth (positive), rather than no growth (death).

One way to perform this test is to rely on the survival time indicated in the lot's certificate of analysis (CoA). According to regulatory requirements, this time defines the window preceding the onset of viable population death; by reaching this point precisely, the expected result is growth at the end of the incubation period.

According to ISO 11138-1, the quality testing of a BI is performed in a resistometer (BIER).[1,3] This equipment develops an extremely rapid heating curve, preceded by an effective air removal stage, which prevents non-condensable gases from obstructing the contact of saturated steam with microorganisms. Under this controlled condition, the accumulated lethality before the exposure phase is minimal. Thus, the survival time provided by the manufacturer strictly reflects the

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Quiz Answers:

Answers: 1. B, 2. C, 3. C, 4. A, 5. B, 6. D, 7. A, 8. C, 9. B, 10. D, 11. C, 12. B, 13. B, 14. B, 15. B

exposure during the cycle's holding phase, which is terminated by rapid depressurization to avoid a residual lethal effect outside the main evaluation phase.

The sterilizers available in CSSDs possess cycle profiles distinct from those observed in a resistometer, particularly regarding the chamber conditioning and depressurization stages. Consequently, these two phases exert a residual lethal effect that is added to the exposure plateau. To correct this distortion and evaluate the process holistically, it is recommended to measure the lethal action of the entire cycle, accounting for all stages involving saturated steam injection, through the lethality calculation (F_0). **Figure 1** illustrates this comparison: when challenging a BI in a hospital sterilizer, it becomes imperative to compensate for the F_0 accumulated during the gradual rise in temperature, whereas the resistometer performs this heating almost instantaneously.

To support the development of these calculations, the use of temperature dataloggers that integrate this mathematical tool is essential. They allow verification of whether the selected exposure time will yield the same dose of lethality indicated in the lot's CoA. Thus, the F_0 values obtained by the physical sensor, which is positioned immediately alongside the tested BIs, must closely approach the survival time established by the manufacturer. Once the attainment of this thermal target is confirmed at the end of the cycle, the vials are sent for incubation.

Currently, thanks to rapid-read fluorescence technology, modern incubators deliver results within a few minutes. In this sublethal test scenario, the expected outcome is the detection of a process failure (positive result for bacterial growth), since the exposure was intentionally restricted to the spores' survival limit. In this manner, the user can track analytical discrepancies with precision, enabling the diagnosis of quality deviations in the indicator lot, issues within the transport and storage chain, or even calibration and operational faults in the incubation equipment itself.

LEARNING OBJECTIVE 2:

Identify practical considerations for assessing BIs based on sterilizer characteristics and monitoring practices

In practice, applying the actions indicated above requires several points of attention because, in certain situations, the expected objective cannot be achieved. Situations in which a test cycle cannot be developed through the sterilizer's

Criterion	Rapid-Readout (Fluorescence)	Traditional Detection (pH Change)
Detection Mechanism	Detects the active state of the alphaglucosidase enzyme, which is naturally bound to the spore coat of viable Geobacillus stearothermophilus spores.	Detects active metabolism. The spore must germinate, multiply, and produce acidic metabolic waste that triggers a pH-induced color change in the
Turnaround Time	Minutes to a few hours (typically ranging from 24 minutes to 3 hours, depending on the manufacturer).	24 to 48 hours (may require up to 7 days for extended incubation in sublethal validation assays).
Turnaround Time	Extremely high for initial enzymatic activity detection near baseline levels	High for macro-scale bacterial population growth and proliferation.
Positive Result Signal	Optical sensors in the incubator detect emitted fluorescent light.	Direct visual color change in the gro\.vth medium (typically shifting from purple/blue to yellow).

Table 1. Comparison of rapid-readout and traditional pH-based growth detection methods. Characteristics summarized from ANSI/AAMI ST79 and the CDC Guideline for Disinfection and Sterilization in Healthcare Facilities.[4,5]

programming, or where a cycle must be manually canceled at a specific stage to simulate a sterilization failure, can contribute to results that lead to misleading conclusions. If not executed correctly, this action is considered a highly unpredictable operation and is prone to diagnostic errors.

If the operator attempts to manually cancel the cycle upon noticing that the sterilizer has reached the survival time on the timer, the result will be inconsistent for two technical reasons:

- **Thermal Inertia and Exhaust Time**: When aborting a cycle, the chamber does not cool down instantly. The pressurized steam takes time to be expelled (exhaust). During these minutes of forced depressurization, the temperature drops gradually, continuing to inject heat (F_0) into the BI vials.
- **Uncontrollable Lethality**: This unplanned residual heat frequently exceeds the spore's resistance. The BI will end up dying (yielding a negative result), leading the user to the mistaken conclusion that the indicator lot is defective, when in fact it was the methodology failure that added extra lethality.

Many professionals believe that if they cannot run a sublethal cycle on the machine, it is sufficient to rely on the BI incubated directly out of the box (unprocessed positive control). However, an unprocessed positive control only proves that the spores are viable and that the culture medium functions correctly. It does not prove whether the spores maintain the proper thermal resistance defined by the D-value. A lot that has undergone thermal degradation during transport may activate normally in a standard positive control yet die prematurely inside the sterilizer during a routine cycle, generating a false-negative (the approval of a contaminated load).

To bypass these issues without causing misleading conclusions, the CSSD should adopt the following strategies:

- **Exclusive Use of Dataloggers with F_0 Software**: Instead of attempting to guess the exact moment to abort the cycle, a physical datalogger should be used to map a short programmable cycle permitted by the machine. The datalogger software retrospectively calculates the accumulated lethality to verify whether the survival target was homogeneously achieved.
- **Test Cycles**: Verify whether the sterilizer manufacturer allows the configuration of a test cycle that primarily includes reduced exposure times, which can be modified in accordance with the survival time of the BI lot being challenged.

By implementing these precautions, errors in test results are avoided, and the design characteristics and technological resources of each sterilizer manufacturer are respected, thereby achieving more reliable and precise outcomes.

LEARNING OBJECTIVE 3:
Compare rapid incubation results with pH-based growth detection and their impact on interpreting BI results in daily use

When evaluating the results of sublethal assays using rapid-read incubators, uncertainties may arise regarding the precision of fluorescence detection, raising questions about equipment calibration, operational failures, or the capability to identify a marginal population of viable microorganisms. To mitigate these doubts and confirm the microbiological growth interpretation, the extended incubation method is recommended. By extending the conventional incubation period, surviving spores are allowed to multiply and consume the nutrients in the culture medium. This active bacterial metabolism results in the production of organic acids that alter the pH of the solution, triggering a visual color change of the growth medium, thereby confirming the microbiological result through two independent detection methods (fluorescence and pH).

The comparison between rapid fluorescence technology and conventional detection via pH-induced color change highlights the balance between the operational agility demanded by modern CSSDs and traditional microbiological safety. The application of each technology directly shapes the regulatory defensibility and logistical efficiency of the hospital; therefore, utilizing pH growth confirmation during BI quality testing becomes vital. **Table 1** compares these two microbiological growth detection techniques, highlighting their basic characteristics and outlining distinct advantages of each method.

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Currently, during the daily use of BIs to monitor sterilization processes in a CSSD, there is no room for extended incubation times. However, the ability to confirm rapid-readout results during product quality testing is fundamental to achieving a correct interpretation of outcomes, thus fulfilling the objective of evaluating the product's true effectiveness.

Conclusion

Validating BIs in CSSDs requires a clear differentiation between routine sterilizer monitoring and quality testing of the indicator itself. Simulating sublethal cycles based on survival time provides a robust scientific tool to audit lots but faces practical challenges in hospital sterilizers due to thermal inertia and software constraints. To mitigate diagnostic errors and misleading conclusions caused by manually aborting cycles, physical dataloggers and the (F_0) lethality calculation are indispensable for compensating for heat accumulated during transition phases. Furthermore, while rapid-read fluorescence technology ensures the logistical agility essential for daily surgical workflows, the traditional pH color-change method through extended incubation serves as an irreplaceable analytical safeguard. Integrating these methodologies strengthens the hospital's regulatory defensibility and the ultimate efficacy of infection prevention and control practices. **HPN**

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Evaluating Biological Indicator Performance in Saturated Steam Sterilization for Central Sterile Supply Department Monitoring - Practice Quiz

1. **What is the primary purpose of using BIs during routine monitoring and process validation?**
   A. To determine the quality of the BI lot
   B. To evaluate the performance of the sterilization equipment and process
   C. To measure the calibration accuracy of the incubator
   D. To verify the chemical composition of the sterilant

2. **How are BI products typically selected for use?**
   A. By comparing growth results from multiple brands during routine monitoring
   B. Based on the performance of lot-specific testing in the CSSD after use
   C. Based on documentation provided by the manufacturer demonstrating compliance with regulatory requirements
   D. By selecting only the BI with the shortest incubation time

3. **Why is it inappropriate to assume that a BI's survival time can be replicated by simply stopping a sterilization cycle when the indicated time is reached?**
   A. BIs cannot be used in hospital sterilizers
   B. The chamber immediately loses all lethality when the cycle is aborted
   C. Residual heat and depressurization continue to contribute additional lethality after cycle cancellation
   D. Survival time applies only to chemical indicators

4. **When conducting a sublethal BI challenge based on the manufacturer's survival time, what result is expected after incubation?**
   A. Bacterial growth (positive result)
   B. A failed incubation test
   C. No bacterial growth (negative result)
   D. Variable results that cannot be predicted

5. **According to the article, why is the lethality accumulated during cycle conditioning and depressurization important when evaluating BI performance in a hospital sterilizer?**
   A. It has no effect on BI results
   B. It can contribute additional lethality beyond the exposure phase
   C. It affects only chemical indicators
   D. It shortens the incubator readout time

6. **What is the purpose of using temperature dataloggers with lethality calculation (F_0) capability during a BI performance evaluation?**
   A. To determine the manufacturer's spore population count
   B. To verify incubator calibration before testing
   C. To replace the need for BI incubation
   D. To confirm that the delivered lethality closely matches the survival time specified in the lot's CoA

7. **When evaluating a BI lot against the survival time specified in its CoA, why is manually canceling the sterilization cycle at the indicated survival time considered unreliable?**
   A. Residual heat and depressurization continue to contribute lethality after cycle cancellation.
   B. The BI cannot be incubated after an interrupted cycle.
   C. The sterilizer immediately enters a validation mode after cycle cancellation.
   D. Steam sterilizers automatically restart the cycle after interruption.

8. **What information does an unprocessed positive control provide?**
   A. That the BI maintains its specified thermal resistance
   B. That the sterilizer is delivering the correct lethality
   C. That the spores are viable and the culture medium functions properly
   D. That the D-value of the BI is within specification

9. **What is the recommended role of a datalogger with F_0 calculation capability when evaluating whether a BI performs according to its CoA?**
   A. To replace the BI during testing
   B. To retrospectively calculate accumulated lethality and verify that the survival target was achieved
   C. To determine the manufacturer's spore population count
   D. To eliminate the need for incubation

10. **What is the primary difference between rapid-readout BI technology and traditional pH-based detection?**
    A. Rapid-readout technology detects bacterial growth, while pH-based detection measures temperature.
    B. There is no difference in the detection mechanism.
    C. Rapid-readout technology uses a color change, while pH-based detection uses fluorescence.
    D. Rapid-readout technology detects enzymatic activity, while pH-based detection detects bacterial metabolism and growth.

11. **Which advantage of rapid-readout technology makes it valuable for routine sterilization process monitoring?**
    A. It requires extended incubation periods. It detects bacterial population growth more effectively than pH-based methods.
    B. It provides results within minutes to a few hours.
    C. It eliminates the need for BIs.

12. **During BI quality testing, why might extended incubation be used in addition to rapid-readout results?**
    A. To verify the sterilizer's temperature calibration.
    B. To confirm microbial growth through pH-based detection.
    C. To determine the BI population count.
    D. To replace fluorescence detection.

13. **What additional factor must be considered when evaluating a BI in a hospital steam sterilizer?**
    A. The BI's incubation temperature
    B. The lethality accumulated during conditioning and depressurization phases
    C. The manufacturer's recommended storage temperature
    D. The sterilizer chamber volume

14. **What is the primary purpose of a BIER resistometer when establishing BI performance characteristics?**
    A. To evaluate routine sterilizer loads in healthcare facilities
    B. B. To provide a controlled cycle that minimizes the influence of non-exposure phases on BI results
    C. C. To replace incubation during BI testing
    D. D. To calculate the F_0 value of hospital sterilizers

15. **What is the primary benefit of evaluating BIs against the performance characteristics listed in the manufacturer's Certificate of Analysis?**
    A. It eliminates the need for routine BI monitoring of sterilization cycles.
    B. It helps verify that the BI lot performs as specified, reducing the risk of misleading sterilization monitoring results.
    C. It determines whether the sterilizer meets installation qualification requirements.
    D. It replaces the need for positive controls and incubation.