Terragene®

Reduced Incubation Time (RIT) Study

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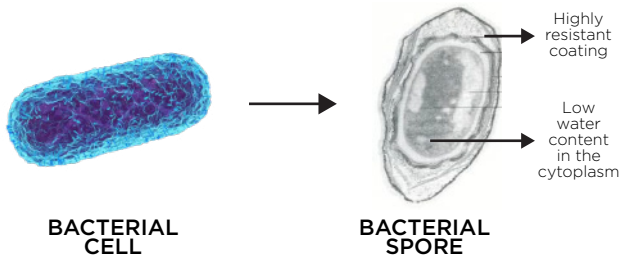
The use of biological indicators (BIs) for monitoring sterilization processes enables the evaluation of the sterilizer's potential to ensure the destruction of the microorganism with the highest resistance to the process. Some bacteria can alter their structure to adapt to unfavourable conditions through a process known as sporulation. During this process, the bacterial cell undergoes various morphological changes that increase its resistance. For instance, the cytoplasm loses a significant amount of water, and since water is an excellent medium for heat conduction, the bacterial cell becomes highly resistant to thermal processes. Additionally, during sporulation, a highly resistant cortex is formed, acting as a protective cover that prevents the penetration of chemical agents. Furthermore, bacterial DNA becomes compacted and is shielded by a protein cover, which reinforces its structure and increases its resistance to the effects of radioactive agents. The operating principle of biological indicators for assessing sterilization processes is based precisely on the presence of these spores.



Bacterial spores require only nutrients and optimal temperature conditions to return to their vegetative state and grow. After undergoing sterilization processes, the spores are placed in contact with a culture medium containing the necessary nutrients and maintained at the appropriate temperature. If any spores have survived, their metabolism will reactivate. When using agar plates as the culture medium, the growth of surviving spores can be observed through visual inspection during a 7-day incubation period.

The main drawback of plate culturing is the need to perform it under strictly controlled conditions in order to prevent cross-contamination. This is essential to avoid false positive results, that is to say, that the observed growth is due to external contamination rather than the survival of the microorganism following the sterilization process.

As an alternative to streamline the development process of BIs, self-contained indicators were developed. In this format, the spore and the culture medium are integrated into a single unit, eliminating the risk of contamination during handling. Moreover, to further accelerate incubation times and enable early detection of bacterial growth, pH indicators were incorporated into specific culture media. During metabolism, spores produce by-products that acidify their environment. This pH change is detected by the indicator and appears as a noticeable color change in the medium. This transformation occurs in significantly less time compared to plate culturing, with detection times of 24, 48, or 72 hours.

To further accelerate results and enable faster decision-making, a new technology was implemented in the field of BIs: fluorescence readouts. Specific spore components were studied to anticipate the results given by changes in the acidity of the culture medium. A particular enzyme was identified that can react with a compound incorporated into the medium to produce a fluorescent by-product. Using an optical system (exciter/receiver), the fluorescence signal generated by the reaction by-product can be detected in a very short time. As a result, response times for BIs have been significantly reduced to 4 hours, 1 hour, 20 minutes, and even as little as 5 minutes.

In urgent situations, assessing the lethality of a sterilization process remained a challenge. To address this issue, Terragene developed a biological indicator based on the fundamental principles of steam sterilization: protein denaturation. This innovative BIs enables the monitoring of steam sterilization processes and delivers results in just 7 seconds, establishing itself as a safe and highly effective tool. To achieve instantaneous results, a structural protein sensor was incorporated into the indicator's culture medium. If the protein is not fully denatured during the process, the sensor binds to specific functional groups of the protein, generating a fluorescence signal. This signal can be instantly detected using a specialized optical system. It serves as evidence that the sterilization process failed to inactivate the microorganism population, as it was unable to completely denature the protein associated with the spore.

Any reading obtained before 7-day incubation period on culture plates is considered a rapid result of the indicator. This result must be validated through a study known as the Reduced Incubation Time (RIT) study.

The FDA's BI Guidance was the first official document to standardize the methodology for this study. In 2021, ISO 11138 introduced Part 8, which specifically addresses this study in relation to indicators designed for monitoring sterilization processes using steam and ethylene oxide.

In summary, both documents converge on a partial cycle identification test, wherein a specified percentage of the exposed indicators must survive the tested conditions. To conduct this study, three

distinct batches must undergo partial cycles, with a minimum of 100 biological indicators per batch. The indicators must be processed and analysed during a 7-day incubation period. The sensitivity of the rapid reading must be greater than or equal to 97%, determined as described below:

$$N_x / N_y * 100 \geq 97\ \%$$

Where:

Nx is the number of positive BIs in the time interval x for the individual partial cycle for each lot (**x** is the reduced incubation time to be validated).

Ny is the number of positive BIs at 7 days of incubation for the individual partial cycle for each lot.

The tests outlined in the FDA biological indicators guide and ISO 11138-8 differ in some points, which are detailed in the table below:

	FDA BI Guidelines	ISO 11138-8
Scope	All BIs with incubation time less than 7 days	Steam and EO BIs with incubation time less than 7 days
Partial cycle	30-80% of the BIs exposed are expected to be positive after 7 days of incubation	30-95% of the BIs exposed are expected to be positive after 7 days of incubation
Conditions for the partial cycle	A partial cycle is one in which all sterilization parameters are met except for the time parameter	Other process variables, such as time, temperature, or concentration, can be adjusted to achieve a partial response
Holding time	No more than 8 hours	It should be documented

The protocol followed by Terragene to validate any reading time other than the standard 7-day period for its BIs adheres to the recommendations outlined in the FDA Biological Indicator Guidance. This document is more comprehensive and encompasses the operating ranges specified in ISO 11138-8. Consequently, our BIs for monitoring steam and ethylene oxide processes feature a reduced incubation time that complies with the recommendations in both ISO 11138-8 and the FDA Biological Indicator Guidance. Meanwhile, the remaining indicators for other processes have rapid readings validated according to the FDA Biological Indicator Guidance.

References

•FDA guidance document 'Biological Indicator (BI) Premarket Notification [510(k)] Submissions', Attachment II, issued on October 4, 2007.

•ISO 11138-8:2021. Sterilization of health care products. Method for validation of a reduced incubation time for a biological indicator.