How to select the ideal biological indicator for specific sterilization needs

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Bacterial spores are the gold standard for monitoring sterilization processes, as they allow for a direct assessment of a method's effectiveness in eliminating pathogens and microorganisms present on a given material. Spores are highly resistant structures produced by certain microorganisms as a defense mechanism in response to adverse conditions.

Through a process known as sporulation, various structural changes occur within the bacterial cell, enabling it to survive under stressful environmental conditions. The bacterial cell loses a significant amount of water from its cytoplasm, which provides high heat resistance. In addition, a protective outer layer is formed, making the spore highly impermeable to chemical agents. The bacterial DNA also becomes tightly compacted, protecting it from damage caused by DNA-disrupting agents such as radiation.

In simple terms, spores behave like seeds that can remain dormant for extended periods until, under favorable conditions, they germinate—returning to a vegetative state and multiplying to form colonies of the original microorganism. This exceptional resilience to harsh conditions is precisely why spores are used in biological indicators (BIs). If a sterilization process can effectively inactivate spores—among the most resistant biological structures—then it should also eliminate all other, less resistant microorganisms, such as vegetative bacteria, mycobacteria, fungi, as well as viruses.

The working principle of a BI is based on its ability to demonstrate whether a sterilization process has reached a sufficient level of lethality to inactivate the spores contained within it. These spores have defined and standardized microbiological resistance. If the sterilization process is successful, the spores will lose their viability, resulting in a negative result. Conversely, if the process is not sufficiently lethal, the spores will remain viable, resulting in a positive result. For this reason, BIs are the only sterilization control methods that provide a direct measurement of process lethality.

According to ISO 11138-8, rapid BIs must be validated with a Reduced Incubation Time (RIT) study. This study ensures that reliable results can be obtained in a shorter time than traditional methods (which typically require 7 days), enabling faster release of sterilized loads.

Given the wide range of products available within Terragene's portfolio, it is essential to determine which BI is best suited for each specific sterilization process. The purpose of this technical document is to explore the different BI formats and then address the key concepts to consider when selecting the most appropriate option for each particular process.

It is important to highlight that all Terragene BIs are designed and validated in accordance with the strict standards of the ISO 11138 guidelines.

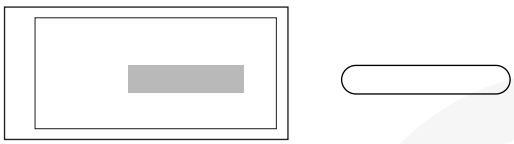
Exploring the different types of BIs

Spore Strips

One of the earliest formats of BIs developed for sterilization monitoring was the spore strip. These consist of a strip inoculated with a spore suspension that is placed inside a protective envelope, which is loaded into the sterilizer along with the items to be sterilized. At the end of the sterilization cycle, the strip must be aseptically removed from the envelope, placed into a specific culture medium, and incubated for 24 to 48 hours to obtain results.

The main drawback of spore strips is the relatively higher risk of false-positive results due to improper handling, which can lead to cross-contamination. Although newer formats have been developed over time to minimize this risk, spore strips are still widely used due to their relatively low cost and its feasibility to evaluate locations with difficult access.

Terragene offers spore strip BIs for various sterilization methods. The materials used for manufacturing the carrier and outer envelope vary depending on compatibility with the sterilization process. As an example, for sterilization processes with steam or ethylene oxide, Terragene offers spore strips with cellulose carriers. Instead, to monitor sterilization processes with hydrogen peroxide, due to the adverse effects of cellulose, the spore strip presentation features a metal carrier fully compatible with the process.

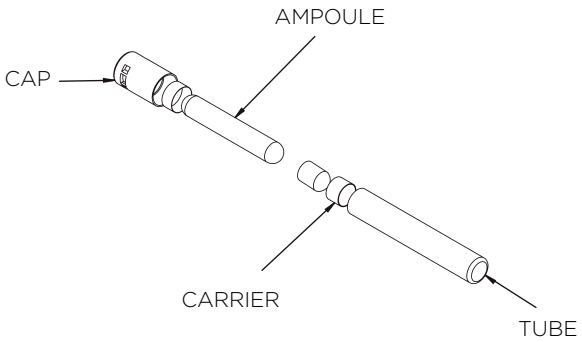


Self-contained biological indicators

Self-contained biological indicators (SCBIs) were developed as an alternative to conventional spore strips in order to reduce the risk of cross contamination caused by handling errors. The

SCBI format includes a spore carrier and a glass ampoule containing culture medium, both housed separately within a single plastic tube. The tube is placed in the sterilizer, and after the cycle is completed, the ampoule is crushed inside the tube, allowing the spores to come into contact with the medium. After incubation at the optimal temperature, results can be observed either through a color change or via fluorescence, depending on the SCBI type.

Terragene offers SCBIs with rapid—even instant—readouts. The fastest results are obtained through fluorescence signals, which can optionally be confirmed through extended incubation to obtain a colorimetric result.



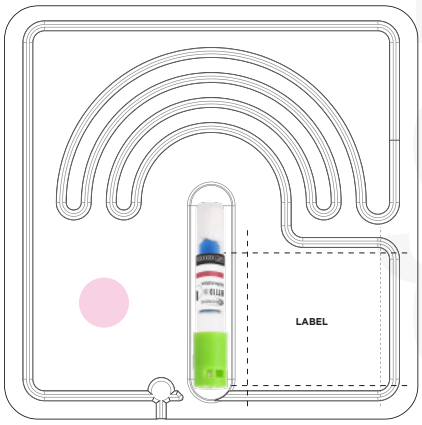
Process Challenge Devices

SCBI formats are also included in a configuration known as a Process Challenge Device (PCD). A PCD is designed to simulate a challenge equal to or greater than that posed by the most difficult-to-sterilize item during routine procedures.

The main advantage of the pre-assembled PCDs developed and marketed by Terragene is their ability to provide a standardized and reproducible challenge in every cycle, enabling reliable evaluation of process performance.

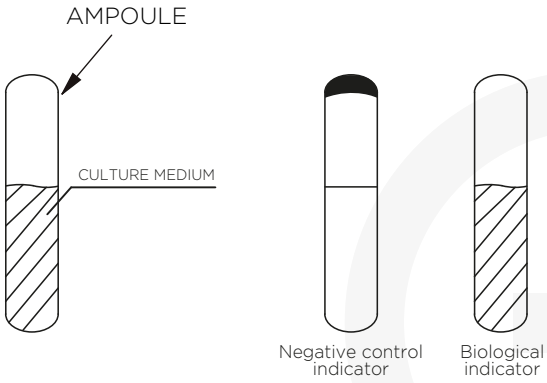
For the monitoring of steam sterilization processes, Terragene offers the PCD presentations known as Steam Packs. These presentations are based on a porous card system that contains different types of biological indicators (with different readout times) and chemical integrators. These systems were validated against the 16-towel pack recommended by AAMI ST79 as the optimal challenge for this type of process.

For monitoring Ethylene Oxide (EO) sterilization processes, Terragene provides a PCD with a "maze-type" design in which the sterilizing agent must travel through a defined path before reaching the BI. This system was validated against the PCD described in AAMI ST41.



Self-contained ampoules

In certain industries, such as the pharmaceutical and food industries, monitoring the sterilization of liquid loads presents a unique challenge, as standard indicators like SCBIs or spore strips cannot be used in such environments. For this purpose, specially designed self-contained ampoules with pre-inoculated culture media are available. These ampoules are placed directly into the core of the liquid to be sterilized. Once the sterilization cycle is complete, the ampoules must be incubated at the optimal temperature for up to 48 hours. Special care must be taken with this format, as it is the only presentation in which the spores are already in direct contact with the culture medium. If the ampoules are exposed to optimal temperatures before processing, the spores may germinate and begin to grow. This would be indicated by a color change in the medium, rendering the ampoule unusable. For this reason, self-contained ampoules must be stored and transported under strict temperature-controlled conditions.



Key aspects in selecting a BI

There is a sheer variety of sterilization methods, and the choice of one over another depends on various factors, such as the type of load to be processed, facility requirements, the toxicity of the sterilizing agent, and its ability to diffuse or penetrate. BIs are specifically designed for each sterilization method, allowing accurate evaluation of the effectiveness of each process. For this reason, the first and most critical step in selecting a BI is to determine which sterilization method will be used.

Steam

Due to its high efficacy, speed, and low cost—as well as the fact that it leaves no toxic residues—saturated steam is one of the most widely used sterilization methods. It is ideal for materials that can withstand heat and moisture, such as stainless steel, textiles, and porous materials. However, it is not suitable for heat-sensitive (thermolabile) materials. Steam acts by transferring energy to the load in the form of latent heat when it condenses on its surface. This heat breaks down the cell wall and denatures cellular proteins. The main parameters to control in steam sterilization are temperature, time, pressure, and steam quality.

ISO 11138-3 recommends using the *Geobacillus stearothermophilus* strain, known for its high heat resistance. Terragene offers a broad range of BIs for monitoring steam sterilization processes, ranging from BT50 and BT60 (spore strip indicators), to the BT20 (a conventional SCBI with visual color change), BT220, BT222, and BT224 (rapid-readout fluorescent indicators), and even the BT225, which provides an instant fluorescent result.

All of these BIs are also available as Process Challenge Device (PCD) kits, which includes a Type 5 chemical indicator. These include: KPCD220-1, KPCD220-2, KPCD220-C, KPCD222-1, KPCD222-2, KPCD222-C, KPCD224-2, KPCD224-C, KPCD225-2, and KPCD225-C.

The nature of the load is another important factor to consider, as Terragene provides specific formats for both solid and liquid loads. For liquids, the BT21, BT22, BT23, and BT24 (self-contained ampoules) were developed. The BT24 contains *Bacillus subtilis* spores and is specifically designed for low-temperature steam processes (110–121 °C).

When sterilizing solid loads, choosing between a SCBI and a spore strip for routine monitoring depends on how quickly results are needed and the accessibility of the point in the process to be evaluated. If fast results are required and you do not have access to an aseptic environment, an SCBI is the best choice. However, if an aseptic environment is available and time is not a constraint, a spore strip offers a more economical option for process monitoring.

It is important to note that an SCBI cannot be placed directly into the sterilizer. It must be inside a pack, a medical-grade pouch, or a PCD.


SPORE STRIPS


CONVENTIONAL BI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PROCESS CHALLENGE DEVICES


AMPOULES

Hydrogen peroxide

Due to its compatibility with heat-sensitive materials and the fact that it leaves no toxic residues, vaporized hydrogen peroxide (VH_2O_2) is widely used as a sterilizing agent. Its sterilizing action is based on oxidation reactions. However, VH_2O_2 has very low penetration capacity, making thorough process control essential. Time, temperature, relative humidity, and VH_2O_2 concentration are the key parameters to monitor to ensure a successful sterilization cycle.

As with steam sterilization, ISO 11138 recommends using the *Geobacillus stearothermophilus* strain for VH_2O_2 process monitoring. When designing indicators for this method, it is crucial to avoid using cellulosic materials, as the peroxide molecule is highly unstable.

Terragene offers a wide range of SCBIs for VH_2O_2 processes: BT91 (conventional readout via color change), and BT95, BT96, and BT98 (rapid readout via fluorescence). As a non-cellulosic alternative to spore strips, Terragene provides BT93/6 indicators, which consist of stainless steel coupons sealed in Plastic/Tyvek® pouches.


SPORE COUPONS


FLUORESCENCE BI


CONVENTIONAL BI

Ethylene oxide

Ethylene oxide (EO) sterilization is widely used for processing materials that are sensitive to heat and

humidity. As a powerful alkylating agent, EO can alter the molecular structure of microorganisms, disrupting their metabolic and reproductive functions and ultimately leading to their death. EO has excellent penetration capabilities, making it ideal for sterilizing loads with lumens and complex or intricate structures. However, special care must be taken, as EO sterilization processes can leave toxic residues if the load is not properly aerated.

EO sterilization depends on four critical parameters: exposure time, temperature, relative humidity, and EO concentration. ISO 11138-2 recommends using Bacillus atrophaeus spores for monitoring EO sterilization processes. Terragene offers a wide range of BIs specifically designed in accordance with ISO guidelines, including BT10 (SCBI with conventional color-change readout), BT110 (SCBI with rapid fluorescent readout), and BT40 and BT60 (spore strips with color-change readout). Terragene has also developed KPCD110 kits (PCD presentation).

PROCESS CHALLENGE DEVICES

FLUORESCENCE BI

SPORE STRIPS

CONVENTIONAL BI

Formaldehyde

Sterilization processes that use low-temperature steam formaldehyde (LTSF) as the sterilizing agent are ideal for treating heat-sensitive materials. The sterilizing action of formaldehyde vapor, like that of EO, is based on its ability to act as a powerful alkylating agent. ISO 11138-5 recommends the use of *Geobacillus stearothermophilus* spores for monitoring LTSF processes. Key parameters to control for successful sterilization include temperature, exposure time, relative humidity, and formaldehyde concentration.

Terragene offers the BT100 (SCBI with conventional color-change readout) and BT102 (SCBI with rapid fluorescent readout), as well as the BT50 (spore strip).

SPORE STRIPS

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Dry heat

Dry heat is one of the simplest sterilization methods, with temperature and exposure time being the key parameters to monitor. This method is ideal for materials that are not damaged by high temperatures, such as glass or stainless steel, as well as substances that are incompatible with steam or moisture, including oils, powders, petroleum jelly, and paraffin. As with EO, ISO 11138-4 recommends using Bacillus atrophaeus spores for monitoring dry heat sterilization processes.

Terragene has developed the BT30 indicator, a conventional SCBI with color-change readout, in which the culture medium and the spore carrier are kept in separate tubes to prevent the medium from evaporating at high temperatures. For dry heat processes, Terragene also offers BT40 and BT60 (spore strips), as well as BT31 (a self-contained ampoule designed to monitor depyrogenation cycles).

SPORE STRIPS

CONVENTIONAL BI

AMPOULES

Conclusion

Selecting the appropriate BI is not merely a technical choice—it is a strategic decision that directly impacts the safety, efficiency, and traceability of sterilization processes. To make an appropriate choice, it is essential to consider not only the sterilization method and the type of load being processed, but also operational factors such as the available time to obtain results and the infrastructure of the sterilization department.

In this regard, understanding the strengths and limitations of each available format, from spore strips to ampoules and rapid-readout SCBIs, allows biological monitoring to be adapted to real-world working conditions, optimizing time without compromising process quality or validation. The correct selection not only ensures compliance with standards such as ISO 11138, but also provides confidence and reliability in the overall process.

The key lies in viewing the BI not as an isolated product, but as part of a comprehensive control system that must effectively and consistently support the specific characteristics of each sterilization process.

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