Terragene®

Speed meets safety

PHOTON®

The technology that is revolutionizing the biological sterilization control systems

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Why is it necessary to sterilize reusable materials?

In the medical field, patient safety and well-being are the paramount priorities. The challenge lies in the constant presence of microorganisms in hospitals and clinics. Improperly sterilized medical materials can become vectors for transmitting bacteria, viruses, and fungi, increasing the risk of healthcare-associated infections (HAIs).

In emergency rooms every second counts, and the pressure can lead to lapses in sterilization control. Without strict monitoring, medical procedures may compromise patient safety. Ensuring successful sterilization is not just a requirement—it is a commitment to life. Adhering to the highest standards at every stage of the process is essential for providing reliable, risk-free healthcare.

When is a material considered sterile?

A material is considered sterile when all living organisms and biological agents have been completely eliminated, inactivated or destroyed. This applies to surfaces, liquids, medications and biological culture media.

Various sterilization methods are used worldwide, with steam sterilization being one of the most common ones. This method combines high temperatures and sustained pressure for a specified period, ensuring sufficient heat transfer to denature microbial proteins and effectively eliminate contaminants.

To verify that sterilization has been effective and that the steam has reached all processed materials, different types of indicators are used:
•***Chemical indicators:*** These change color when exposed to the sterilizing agent or under specific process parameters, providing an immediate visual confirmation.
•***Biological indicators:*** These contain highly resistant spores that should be inactivated during sterilization. Unlike chemical indicators, their verification takes longer, as spore growth must be assessed to confirm cycle efficacy.

In hospital environments, where the demand for sterilized supplies is constant, release time plays a crucial role. The ability to use a sterilized medical device depends on the results of the indicators monitoring the process. Therefore, rapid visualization options are essential to optimize the time without compromising patient safety or procedural effectiveness. While rapid fluorescence-based reading technologies have significantly reduced the time required to obtain biological indicator results, many still do not provide the immediacy required in critical emergency situations.

Photon: The best option for immediate and reliable sterile release

To enable the early and safe release of sterilized loads from autoclaves, Terragene has developed the fastest system on the market: the Bionova® Photon Auto-Reader incubator, also known as Photon, in combination with the Bionova® Photon BT225 biological indicator. With an automatic reading in just 7 seconds, Photon is an unprecedented system that delivers instant, reliable, and easy-to-interpret results.

The Bionova® Photon BT225 biological indicator contains *Geobacillus stearothermophilus* spores, and its culture medium includes a protein-sensing compound that generates fluorescence. This product has been validated for both vacuum-assisted and gravity displacement sterilization cycles operating at 132–135°C and was designed in compliance with ISO 11138-1:2017 and ISO 11138-3:2017 quality standards.

In the classic fluorescence readout SCBIs, in those SCBIs exposed to an inefficient (or unexposed) sterilization cycle, the active α-glucosidase enzymes, which are naturally located in different regions of the *Geobacillus stearothermophilus* spore structure, catalyze the breakdown of the nonfluorescent α-MUG substrate, releasing the 4-MU product. When this product is excited by UV light generated by the autoreader (exc = 340-380 nm), it emits a fluorescence signal that is detected by the autoreader itself (em = 455-465 nm).

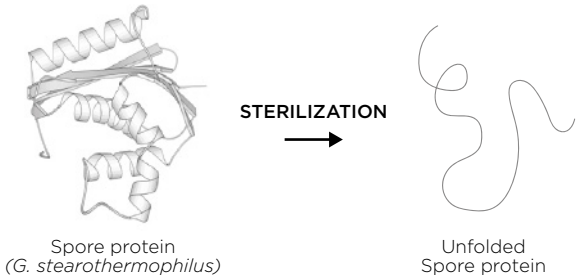
Bionova® BT225, on the contrary, relies on the instant interaction between the fluorophore (ANS) and the hydrophobic regions of the spore proteins. When exposed to a successful sterilization cycle, these regions are destroyed due to protein unfolding. This disruption impacts the fluorescent properties of ANS, reducing fluorophore emission, as it can no longer bind to the protein, resulting in very low fluorescence.

How does Bionova® Photon BT225 work?

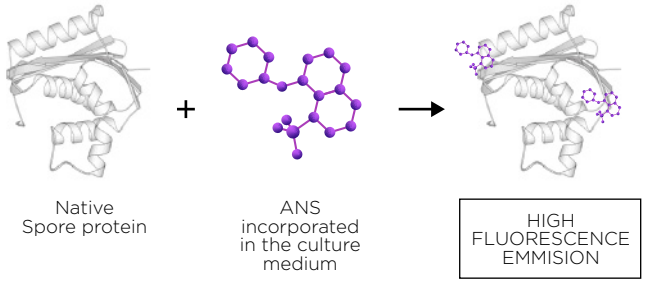
ANS (1-anilinonaphthalene 8-sulfonate), the distinguishing component between Bionova® BT225 device and previous versions of the biological indicator, is a fluorescent probe frequently used to monitor conformational changes in proteins. It can

bind noncovalently to native and partially unfolded proteins specifically at the transmembrane domain. Its fluorescent features vary with changes in the probe environment. When the sensor binds to the protein, specifically to hydrophobic pockets in its structure, there is a shift in light/energy emission within the blue light spectrum.

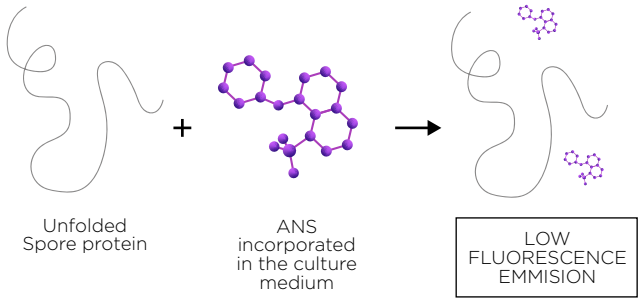
As the sterilization process is carried out, outer proteins present in the spores inoculated on the SCBI carrier are denatured and their tridimensional structure is modified moving into a permanent unfolded state. That final tridimensional structure is strongly related to sterilization process conditions such as temperature, moisture and exposure time. Therefore, these outer proteins could be regarded as "sensor proteins", since their conformation can be modified by the sterilization cycle conditions.



Upon the SCBI activation (the ampoule contained in the SCBI is crushed), the culture medium soaks the carrier and the ANS comes into contact with the spores (and their outer proteins). In non-sterilized SCBIs or after unsuccessfully sterilization processes, the ANS molecules bind to the hydrophobic cavities in outer proteins, significantly increasing its quantum yield, allowing to detect this interaction by fluorescence techniques:



In contrast, when the SCBI is subjected to a successful sterilization process, spore proteins suffer an irreversible conformational change. This unfolding destroys the hydrophobic pockets to which the fluorophore binds. In this way, the fluorophore remains in contact with the aqueous medium (polar environment), leading to a substantially lower fluorescence yield than when interacting with the structurally intact hydrophobic pockets:

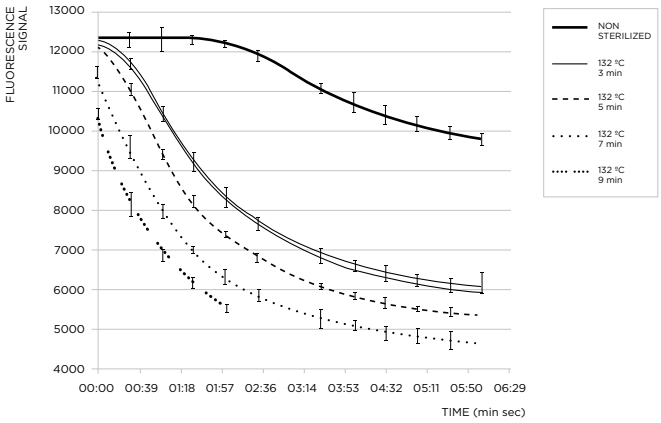


This ANS's sensory ability is an instantaneous phenomenon that can be detected immediately, instead of needing the time for enzyme catalytic activity.

Consequently, since unfolding of spore proteins is directly correlated with a successful sterilization process, and, therefore, with death of the microbial spores, determination of the efficacy of the sterilization process is available instantly.

After 7 seconds, Bionova® BT225 when used with Bionova® Photon Auto-Reader Incubator (BPH), yields a detectable fluorescence signal. The SCBI is exposed by the incubator to UV light at 340-380 nm and the associated emission from the fluorophore (ANS molecules bound to protein hydrophobic pockets) is captured in the 455-465 nm range. The detection of fluorescence signal above a certain threshold upon incubation of BT225 SCBI in the Bionova® Photon Auto-Reader Incubator (BPH) indicates a sterilization failure.

The following graph illustrates the evolution of the fluorescence signal in Bionova® Photon BT225 indicators that were not exposed (i.e., did not undergo the sterilization process), measured at different incubation and reading times. It also displays the fluorescence signal of indicators exposed to sterilization cycles at 132°C for varying durations (3, 5, 7, and 9 minutes).



When comparing the emitted signals, it is evident that the more lethal the sterilization cycle (i.e., the longer the exposure time), the lower the fluorescence signal. Additionally, a clear

distinction can be observed between the fluorescence signal of unexposed indicators and those subjected to different sterilization cycles.

The BT225 SCBI can also indicate the presence of *G. stearothermophilus* organisms by a visual pH color change reaction. Biochemical activity of the *G. stearothermophilus* organisms produces acidic metabolic by-products that cause the media to change color from purple to yellow, which also indicates a sterilization process failure. This optional confirmation can be done by incubating the SCBI for 48 hours at 60 °C.

According to the FDA guidance document "Biological Indicator (BI) Premarket Notification [510(k)] Submissions" and ISO 11138-8:2021 "Sterilization of health care products - Biological indicators - Method for validation of a reduced incubation time for a biological indicator", the 7-second readout has been additionally correlated with the 7-day visual pH color change result. Bionova® BT225 has a 7 second reduced incubation time result that correlates to the 7 day (168 hours) visual readout with a sensitivity ≥ 97 %.

Sensitivity of the instant reading in Bionova® Photon BT225

Any rapid biological indicator predicts and anticipates the spore growth in a 7-day conventional culture. The sensitivity and accuracy of Bionova® Photon BT225 have been validated in accordance with ISO 11138-8:2021 and the FDA Biological Indicator Guidance (*Attachment II of the "Biological Indicator (BI) Premarket Notification [510(k)] Submissions"*).

During the Reduced Incubation Time (RIT) study, Bionova® Photon BT225 indicators were exposed to sublethal conditions, following the guidelines of the aforementioned standards. These conditions were carefully selected to ensure that 30–80% of the indicators survived the process. The table below presents the conditions used for RIT validation:

Cycle	Temp (°C)	Time (sec)	Type
1	132	135	Vacuum-assisted
2	135	90	Vacuum-assisted
3	132	75	Gravity air displacement

Three batches of indicators (100 per batch) were tested under each sublethal condition. A 7-second rapid reading was conducted with the Bionova® BPH Instant™ incubator, while spore growth was monitored over 7 days of incubation, with observations recorded every 48 hours.
The system's sensitivity is calculated using the following expression:

$$\text{Sensitivity} = \frac{\text{Number of Growth Positives after 7 days - Number of False Negatives}}{\text{Number of Growth Positives after 7 days}} \times 100$$

A false-negative indicator is one that initially produces a negative instant reading but later shows growth during the 7-day extended incubation.

The tables below show the results obtained during the RIT studies of Bionova® Photon BT225:

Calculation of Sensitivity - Exposure cycle: 132°C, 135 seconds (vacuum-assisted)

Batch	Number of Positive BIs after 7-day incubation	Number of Positive BIs after 7-sec incubation	False negatives after 7-sec incubation	Sensitivity at 7 sec (%)	Number of Positive BIs after 48-h incubation	False negatives after 48-h incubation	Sensitivity at 48 h (%)
A	56	68	0	100	56	0	100
B	42	49	0	100	42	0	100
C	51	60	0	100	51	0	100

Calculation of Sensitivity - Exposure cycle: 135°C, 90 seconds (vacuum-assisted)

Batch	Number of Positive BIs after 7-day incubation	Number of Positive BIs after 7-sec incubation	False negatives after 7-sec incubation	Sensitivity at 7 sec (%)	Number of Positive BIs after 48-h incubation	False negatives after 48-h incubation	Sensitivity at 48 h (%)
A	62	71	0	100	62	0	100
B	49	57	0	100	49	0	100
C	58	65	0	100	58	0	100

Calculation of Sensitivity - Exposure cycle: 132°C, 75 seconds (gravity air displacement)

Batch	Number of Positive BIs after 7-day incubation	Number of Positive BIs after 7-sec incubation	False negatives after 7-sec incubation	Sensitivity at 7 sec (%)	Number of Positive BIs after 48-h incubation	False negatives after 48-h incubation	Sensitivity at 48 h (%)
A	62	70	0	100	62	0	100
B	49	56	0	100	49	0	100
C	58	67	0	100	58	0	100

According to ISO 11138-8 and the FDA biological indicator guidance, a rapid reading is considered reliable when RIT studies yield sensitivities above 97%. Studies conducted with Bionova® Photon BT225 demonstrate that its 7-second reading is sensitive enough to ensure safe load release.

The Photon system combines groundbreaking speed and precision, offering an efficient solution for optimizing sterilization processes without compromising patient safety. Its high sensitivity and instant results make it a powerful tool for making immediate and reliable decisions in critical medical situations.

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