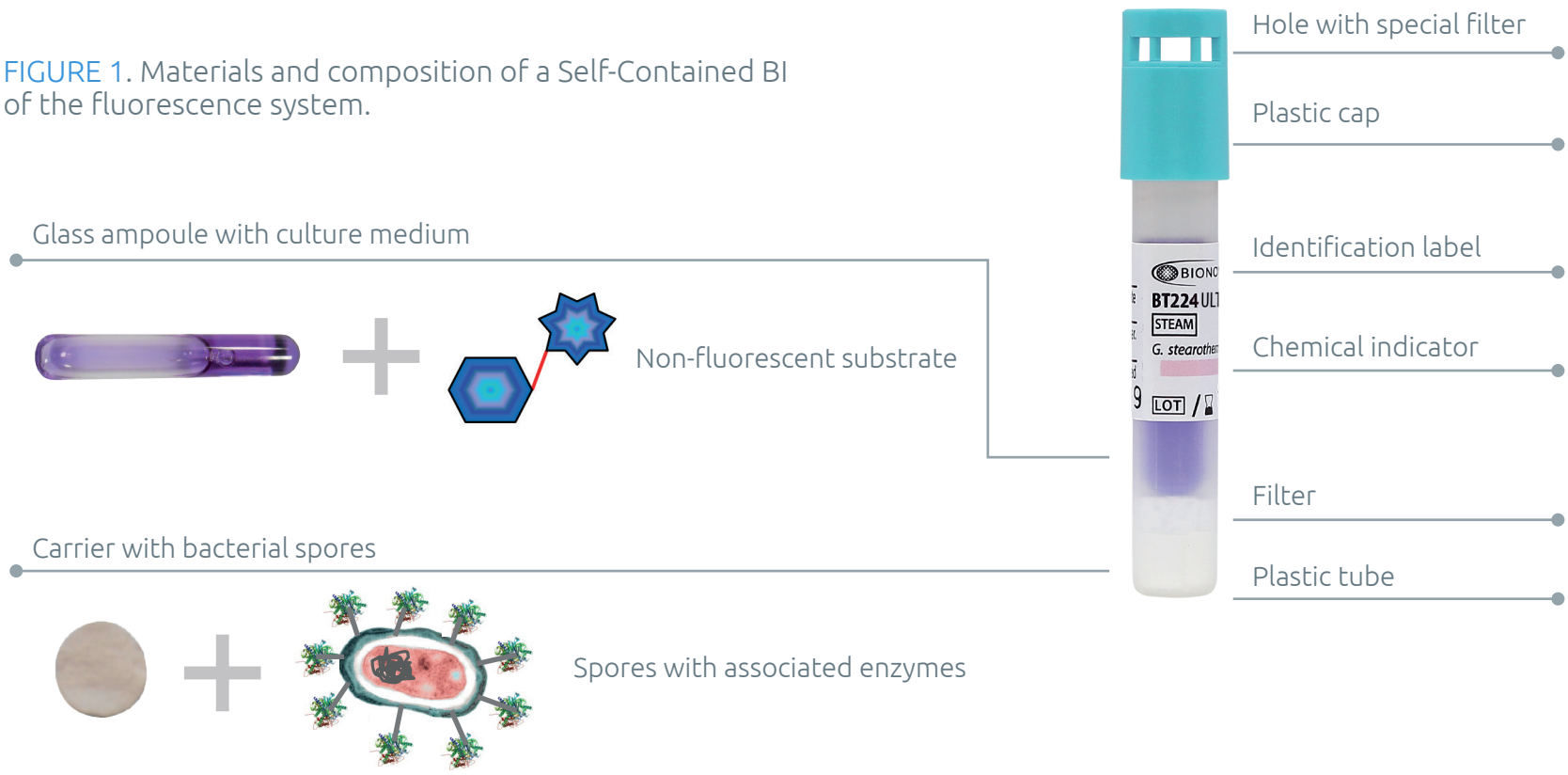
HOW DOES **FLUORESCENCE READOUT** WORK?

The answer is: thanks to an enzymatic reaction that predicts the spore viability.

The answer is: thanks to an enzymatic reaction that predicts the spore viability.
For an enzymatic reaction to occur, we need two key players: an enzyme and its substrate (or a compatible modified substrate). In Bionova® Fluorescence system, the enzyme is associated to the spore coating (accumulated during sporulation, in other words, the spore formation) and the substrate is included in the synthetic culture medium provided in the sealed glass ampoule of the BI (see Figure 1). This substrate corresponds to a non-fluorescent carbohydrate that will convert into a fluorescent by-product after the glucosidase activity of the spore-associated enzyme (see Figure 2). Depending on the bacterial species included in the BI will be the corresponding enzyme: α-glucosidase for *Geobacillus stearothermophilus* and β-glucosidase for *Bacillus atrophaeus*. It is worth mentioning that our BIs do not contain any GMO (Genetically Modified Organism) as the species abovementioned correspond to the original ATCC bacterial culture. Moreover, we do not add any external enzymes and the system relies exclusively on the enzymes naturally produced and associated to the spores.
Enzymatic reaction occurs when the ampoule is broken, enzyme and substrate come together (and if the enzyme activity was not destroyed with a sterilization procedure). Following the enzymatic activity, the fluorescent by-product will be produced. Fluorescence will be detected by the readout system included in Bionova® auto-readers, which are design for this purpuse.

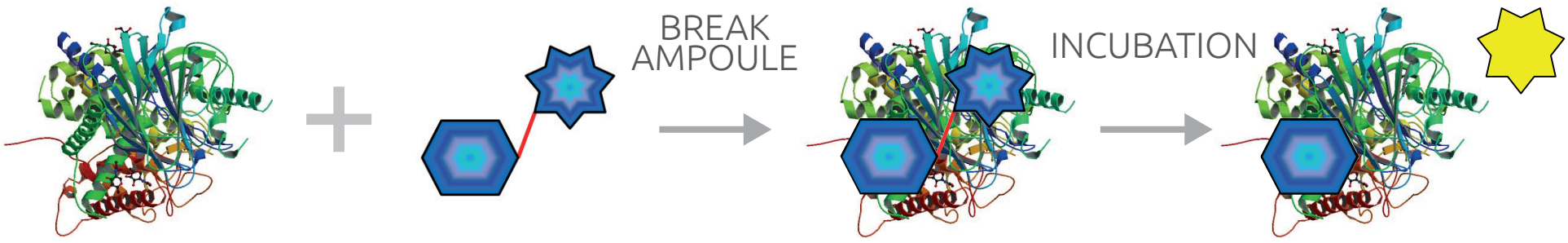
FIGURE 1. Materials and composition of a Self-Contained BI of the fluorescence system.



Bionova® Auto-Reader Incubator's line.

ENZIMATIC REACTION

FIGURE 2. Fluorescence system enzymatic reaction.

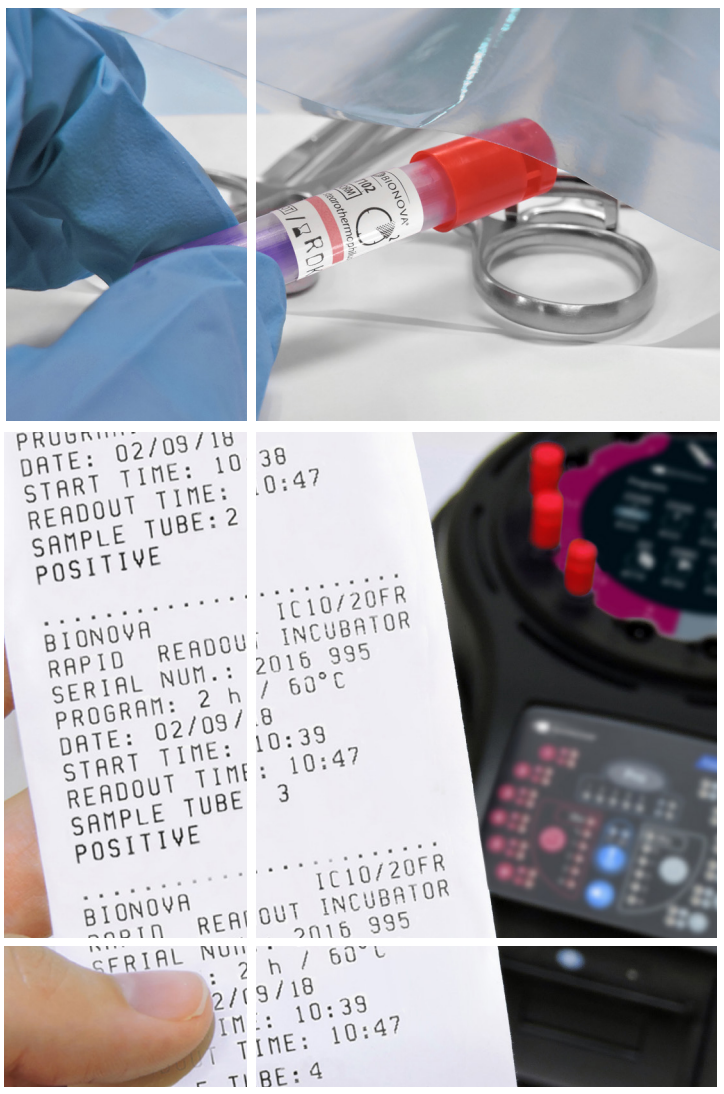


α-glucosidase (*G. stearothermophilus*)
β-glucosidase (*B. atrophaeus*)
Spore-associated enzymes

Non-fluorescent substrate
Incorporated in the culture medium

Substrate recognition
Enzymatic reaction occurs

Fluorescent by-product formation
Fluorescence is generated



In Figure 3 it is summarized the different possible results it can be obtained when a BI is exposed to a sterilization cycle. After being removed from the sterilizer, the cap is sealed and the ampoule broken. If the cycle is sub-lethal, spores will remain alive and the enzymes mentioned aboved will maintain its activity. When the surviving spores get in contact with the culture medium, they will start to germinate and enzymatic reaction will happen, giving a fluorescence signal. This fluorescence will be detected by special sensors inside the wells of the incubator/auto-reader devices, informing the user that the result is **positive** hence pointing to a failure in one or a combination of sterilization parameters.
If the stylization cycle is lethal, no live spores will remain and their associated enzymes will not remain active either. This means that no fluorescent by-product will be generated; no fluorescence will be detected by the incubator/reader, thus giving a **negative** result. This is interpreted as a successful sterilization cycle regarding its sterility.

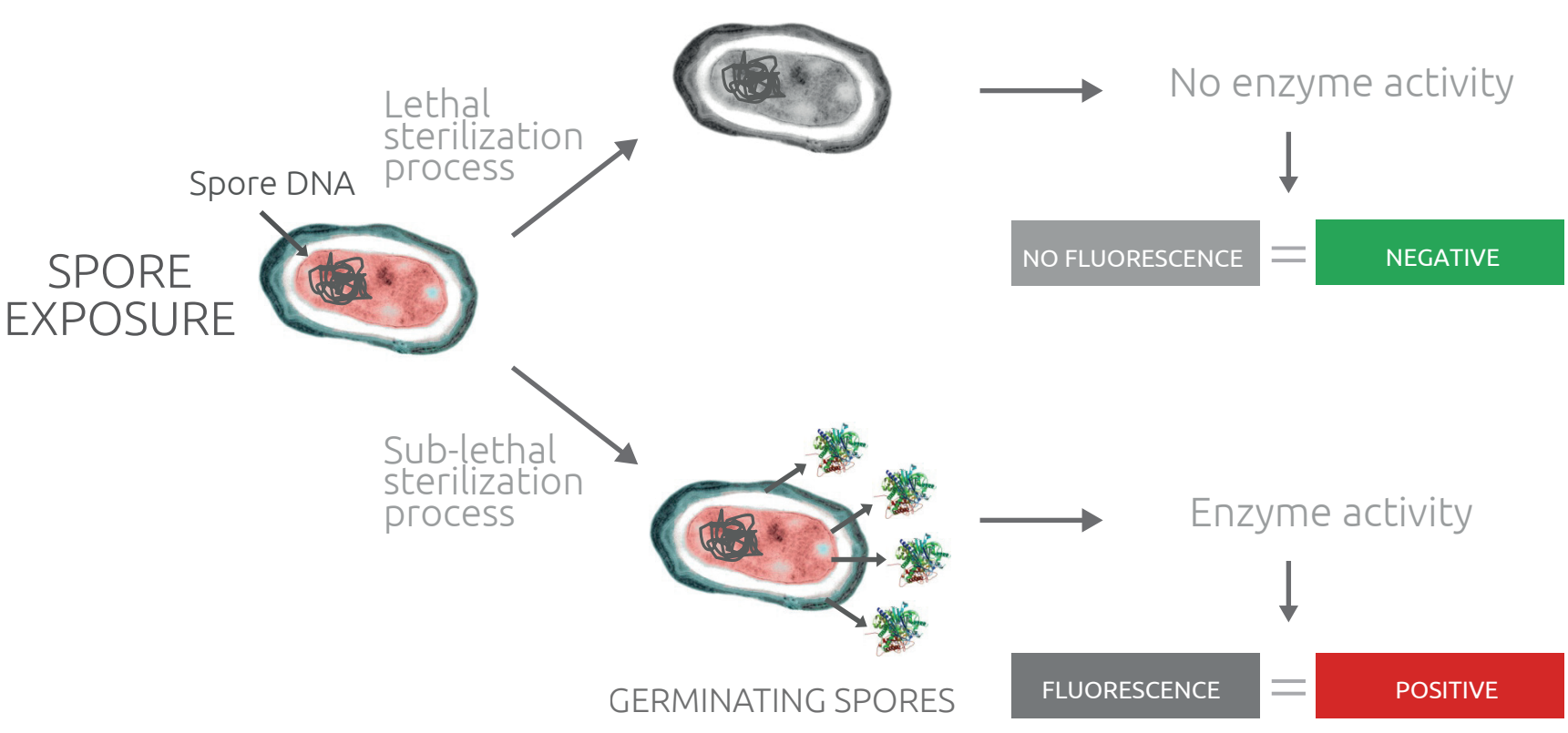
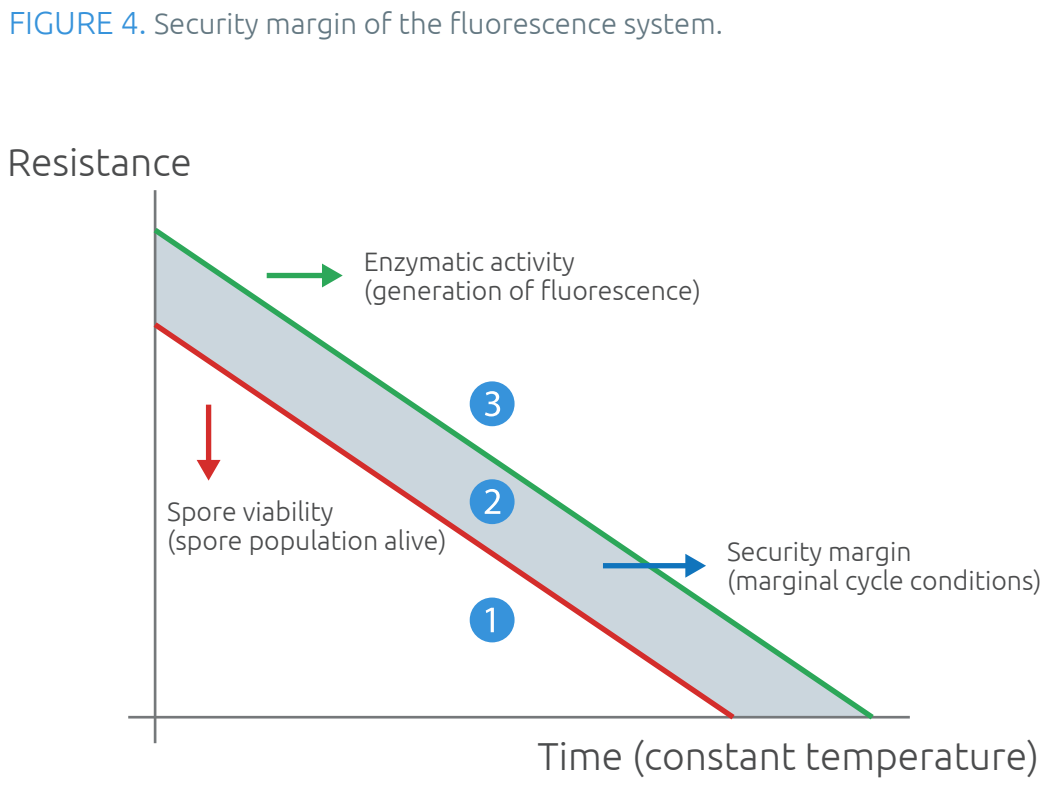


FIGURE 3. Lethal vs. Sub-lethal sterilization processes and how are they detected by the fluorescence BI system.

It is very important that the user keeps in mind that this system predicts the results with a high sensitivity. Related to this, the FDA requires the sensitivity of the reduced incubation time to be greater than 97% (prediction of the fluorescence system compared with the conventional one). According to Annex II of “Biological Indicator (BI) Premarket Notification [510(k)] Submissions” from FDA.
Another important feature of these fluorescence systems is its Security Margin, as illustrated in Figure 4. This safety attribute of the system relies on the fact that the enzyme´s biological activity is more resistant than the spore viability. In doing so, the system avoids the possibility to obtain a False Negative result (negative fluorescence but positive growth), which could imply a very dangerous risk to the sterility of the process. This means that when a sterilization cycle lies in a margin of sterility, e.g. if we have an overloaded chamber, the system will be able to distinguish and detect it, giving a rapid fluorescence positive result but a negative spore outgrowth (after extended incubation). This result should be considered as a sterilization process failure and the appropriate actions should be taken. Consequently, the activity of spore-associated enzyme provides a higher sensitivity to an inadequate or marginal sterilization process than the spore´s resistance itself.

FIGURE 4. Security margin of the fluorescence system.



1. Positive fluorescence (enzymatic activity), positive growth (spores remaining)
2. Security margin (to avoid false negative): positive fluorescence (enzymatic activity), negative growth (all spores dead).
3. Negative fluorescence (extinguished enzymatic activity), negative growth (all spores dead).

As the user can appreciate, development of this type of predictive system is undoubtedly an opportunity without precedents, which has arisen a race towards the fastest BI in the market. This will be translated not only in quicker instrument turnover and safer sterilization monitoring but, also, will imply a decrease in hospital investment in reusable instruments and devices. For this reason, fluorescence readout system has given the chance for BIs to come into sight again after a long period of being behind the scene of Chemical Indicators, when readout times or BI complex handling left BIs in the shadows.
Altogether, fluorescence readout technology has led to a change in CSSD paradigms. If you are interested in learning more about this change of paradigms, click here.