Create a better future

Re-incubation of fluorescence readout Biological Indicators: a common mistake

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During the last decade, the industry of biological indicators has faced new challenges; such as the need of a faster reusable materials' reprocessing, the development of new medical devices and instruments, the emergence of new technologies for their reprocessing, and the need for new indicators appropriate for their control.

In the face of these new challenges, manufacturers of products for sterilization control have invested their efforts in the development of a new range of rapid readout biological indicators, based on fluorescence as an early detection method, providing sterilized instruments in less time.

Fluorescence is a short-term luminous phenomenon, undetectable to the human eye, through which substances with the capacity to fluoresce absorb energy, get "excited" and subsequently radiate light, that is to say, they fluoresce (see fig. 1). Each fluorescent molecule is able to absorb and radiate energy with certain characteristics, which allow their stimulation and detection through special filters and sensors (for example, in the reading position of an auto-reader incubator).

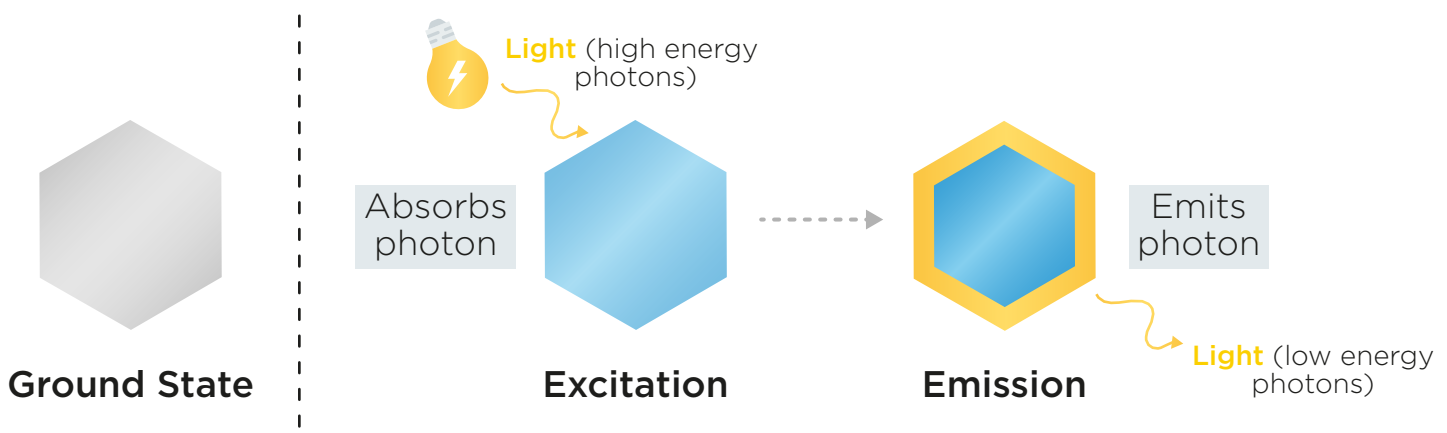

Figure 1. Graphic representation of what happens when a molecule able to fluoresce is stimulated with the proper energy.

Terragene®, pioneer in technological innovation for infection control, developed biological

indicators based on the fluorescence system when the inoculated spores remain viable after the sterilization process, significantly reducing the incubation times.

The fluorescence's early detection system is based on an enzymatic activity that derives from an enzyme (protein with intrinsic activity) found on spores. These enzymes will be available at the moment in which the spores get in contact with the culture medium, are hydrated and germinate. At such moment, the enzymes will start to act on the fluorescent substrate that is in the culture medium.

What happens since the union between the substrate and the enzyme is going to determine how the BI behaves, and it is described by the reaction kinetics. In other words, the appearance, permanence and disappearance of fluorescence depend of the enzymatic activity.

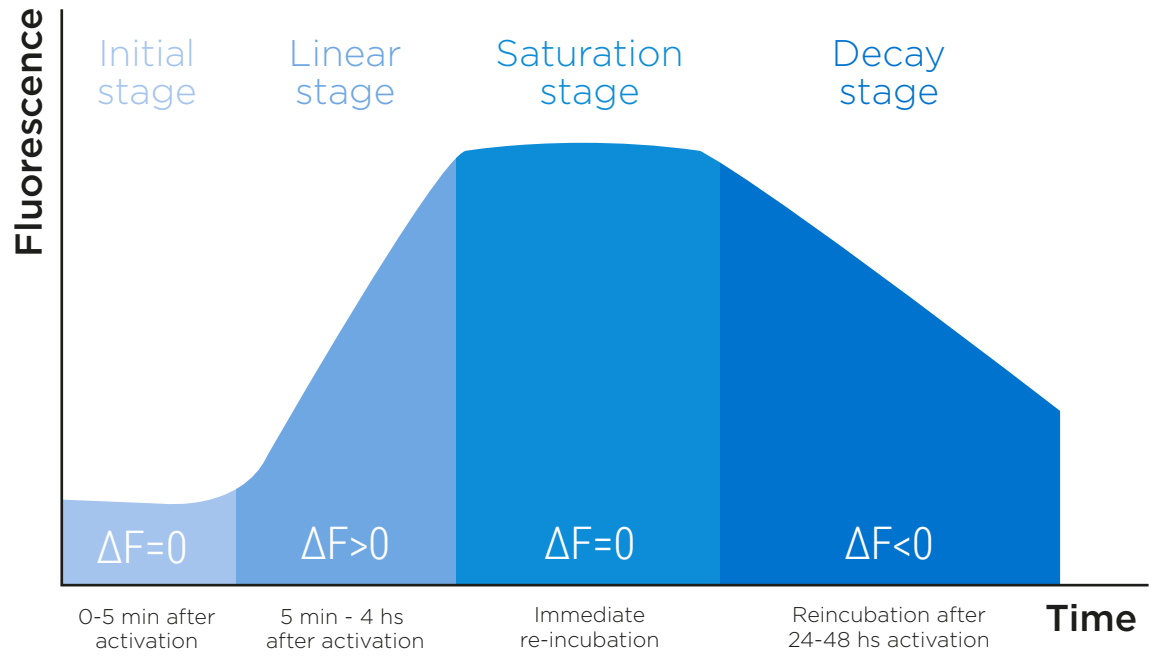

Figure 2. Fluorescence Readout system's curve of enzyme kinetics. ΔF corresponds to the difference between the values measured at a certain point of the incubation regarding the values taken as reference during the initial stage.

The enzymatic reaction is a dynamic process, and because of this, it is not possible to know a priori the exact moment in which fluorescence will emerge. The moment and intensity of the fluorescence will depend on the quantity of enzymes available that are active after the sterilization process, on the status of the culture medium after the exposition, and on the incubation temperature, among others. Therefore, in order to detect fluorescence in the shortest possible time and to determine if the result is positive or negative, fluorescence should be measured throughout the whole incubation process relativizing each measurement to a reference value; that is to say, the measurements correspond to the differences between the measured value and the reference value (it is different for every BI). When that difference is bigger than a pre-established value for the BI under consideration, the result will be positive, and this could happen at any time during the incubation period. When the difference is below the reference value, the result will be negative at the end of the program.

The fluorescence BI's curve of enzyme kinetics consists of four clearly defined phases that match the fluorescence emission in case there are viable spores after the sterilization process. If no spore survives the sterilization cycle, there will be no germination nor growth, no fluorescence nor enzymatic activity. In this case, the result will inevitably be negative.

The **initial** or latency stage is the necessary time for spores to get in contact with the culture medium, to notice the favorable conditions it gives them, to reach the minimum germination temperature and to produce actual germination process. At this stage there is no fluorescence, since there are not yet any enzymes available to react with the substrate and generate fluorescence ($\Delta F=0$), and therefore, **the auto-reader incubator will not produce any result**. This is one of the main reasons why biological indicators need a minimum incubation time. At this stage, the fluorescence's reference value is taken, in order to make the subsequent comparative measures.

At the **linear** stage, the speed of reaction is the highest, since the enzymes get in contact with the substrates in the culture medium, giving place to notable increases of fluorescence ($\Delta F>0$). At this point, the auto-reader performs fluorescence readouts during the period corresponding to each BI program, or until it detects a difference between them, **giving a positive result.**

During the **saturation** or stationary stage, there is no net enzymatic activity since all the enzymes are occupied with substrate, therefore there is no net increase of fluorescence ($\Delta F=0$). If a BI is incubated and you try to read it during this stage of enzymatic activity (for example, by re-incubating the BI), **the result given by the auto-reader will be negative**, because the fluorescence's initial reference value will be at its maximum, and therefore, the incubator will not detect any difference along the incubation. The result will be negative even when the indicator has given a positive result during its first incubation (when the reference value was the one corresponding to the enzymatic activity's initial stage).

During the inhibition or **decline** stage ($\Delta F<0$), the slope is linear and negative, since the substrate begins to run out, and/or the inhibition phenomenon occurs due to an excess of product. If an indicator at this point of the stage is re-incubated, as may happen the day after the initial incubation, **the result given by the auto-reader will be negative** (the enzyme is inhibited or there is no more substrate to process).

For this, **under no circumstances you should re-incubate biological indicators based on fluorescence.**
A BI based on fluorescence readout **must be incubated immediately after its activation.**

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