Create a better future

Biological indicators
Quality parameters

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Biological Indicators (BIs) are the main alternative to ensure the effectiveness of the sterilization processes. They are considered the highest level of sterility assurance because they actually test the sterilizer ability to kill specific strains of highly resistant organisms. Microorganisms widely recognized as suitable for BIs are bacterial spores because they are significantly more resistant than normal microflora.

Since *Geobacillus stearothermophilus* spores demonstrate a high resistance toward steam, formaldehyde and vaporized/plasma hydrogen peroxide, they're used in biological indicators that monitor these sterilization processes, while BIs with *Bacillus atrophaeus* are recommended for ethylene oxide and dry heat applications. *Bacillus subtilis* and *Bacillus pumilus* are used for controlling steam sterilization process at low temperature (110-121°C) and gamma irradiation sterilization processes respectively.

Each BI contains viable spore population with a defined resistance to a specific sterilization process. The **population size (P_0)** is expressed in terms of colony-forming unit (CFU), a unit used to estimate the number of viable bacteria cells in a sample. When we talk about viable bacteria cells, we are referring to the bacteria cells ability to multiply via binary fission under the controlled conditions.

Terragene® designs and validates all BIs according to ISO 11138:2017 specifications. This standard specifies the quality parameters that should govern the BI performance. In addition to the population size, ISO 11138:2017 define the resistance of the indicator through the **D-Value**. The D-value for a BI is the time (or dose) at a specified set of exposure conditions that will result in a 1 Log (or 90%) reduction to the viable spore population. The D-value is a measure of spore resistance to a particular sterilization process: the higher the D-value, the more resistant the microorganism is to destruction. ISO 11138:2017 Annexes C and D suggest different methods for the D-Value calculation. Terragene® calculates this quality parameter by a fraction negative method: the Holcomb-Spearman-Karber Limited (HSKL) Method.

With the population size and the D-value, two quality parameters can be calculated: the survival time and the kill time.
The **survival time** is the exposure time required for all BI's of a defined population in order to retain viable spores According to ISO11138:2017 it is possible to calculate the survival time as:

$$\text{Survival time} = \text{Dvalue} * [\log(P_0) - 2]$$

The “-2” removes 2 log reductions before a spore count of 1 is achieve, leaving 100 spores per BI. This gives a high chance of growth on all BIs.

The **kill time** is the exposure time required to achieve inactivation of the BI population to the extent that the probability of a viable biological indicator being 1 in 10 000. According to ISO 11138:2017 it is possible to calculate the kill time as:

$$\text{Kill time} = \text{Dvalue} * [\log(P_0) + 4]$$

The “+4” adds 4 further log reductions beyond a spore count of 1 spore per BI.

Another quality parameter used is the **Z-Value**. This parameter is associated with thermal sterilization processes, and its formal definition is the change in the exposure temperature that change 10 times the D-value. The Z-value is an indicator of the BI sensitivity to thermal treatments. To calculate the Z-Value, the D value is evaluated at three different temperature. This D-value vs temperature data in a log-linear regression analysis and from a graphical perspective, the Z-value is the negative reciprocal of the slope of the line of best fit.

ISO 11138:2017 also sets the possible minimum values of these quality parameters. A summary is presented in the table below:

STEAM (ISO 11138-1 and ISO 11138-3)	
POPULATION	≥ 1 exp 5 = 100.000 spores *(Geobacillus stearothermophilus)*
D-VALUE	≥ 1.5 minutes
Z-VALUE	≥ 6°C
EO (ISO 11138-1 and ISO 11138-2)	
POPULATION	≥ 1 exp 6 = 1.000.000 spores *(Bacillus atrophaeus)*
D-VALUE	≥ 2.0 minutes
FORM (ISO 11138-1 and ISO 11138-5)	
POPULATION	≥ 1 exp 5 = 100.000 spores *(Geobacillus stearothermophilus)*
D-VALUE	≥ 6.0 minutes
H202 (ISO 11138-1)	
POPULATION	≥ 1 exp 5 = 100.000 spores *(Geobacillus stearothermophilus)*
DRY (ISO 11138-1 and ISO 11138-4)	
POPULATION	≥ 1 exp 6 = 1.000.000 spores *(Bacillus atrophaeus)*
D-VALUE	≥ 2.0 minutes
Z-VALUE	≥ 20°C