Resistance parameters: Application of population, KT, ST, and D-Value

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After every sterilization procedure, it is crucial to verify that the selected method has effectively eliminated all potential microorganisms. For this reason, sterilization processes must be monitored using both biological and chemical indicators.

Biological indicators (BIs), or spore tests, are the most widely accepted method for assessing sterilization effectiveness, as they represent the highest level of challenge: BIs assess the sterilization process by using microorganisms that are highly resistant to specific adverse conditions. In other words, sterility assurance is based on the process's ability to inactivate specific bacterial spores, which are used due to their higher resistance compared to other typical microorganisms.

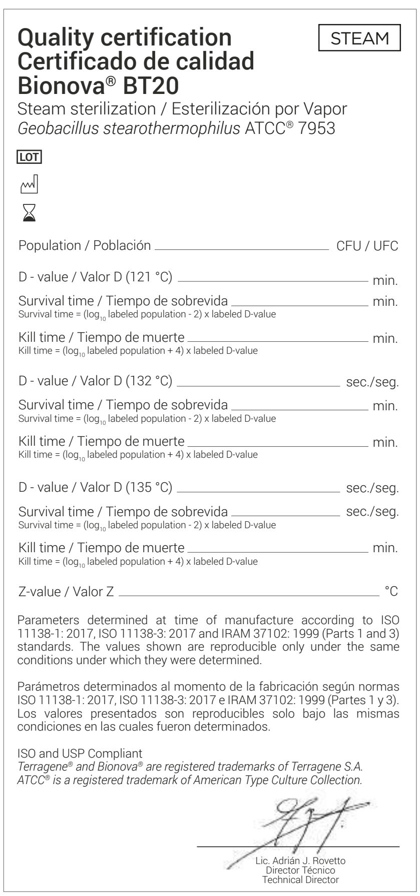
However, how can it be ensured that the process effectively kills the spores contained in the BI? Certain quality parameters help determine whether sterilization conditions have been successfully achieved.

The purpose of this technical content is to explain the quality parameters listed in the certificate of analysis (COA), particularly the D-Value, kill time (KT), survival time (ST), and Z-Value, which are batch-specific. According to ISO 11138, manufacturers must provide information on these parameters for each batch, as they are essential for understanding the behavior and resistance of BIs.

Definitions

Certificate of analysis (COA)

The certificate of analysis (COA) is a document that provides detailed information on the quality and safety of a product. This document is issued for each specific batch and includes the values of key parameters required for the product to meet the criteria established by regulatory bodies.



Quality certification
Certificado de calidad
Bionova® BT20 STEAM

Steam sterilization / Esterilización por Vapor
Geobacillus stearothermophilus ATCC® 7953

LOT

Population / Población ______ CFU / UFC

D - value / Valor D (121 °C) ______ min.

Survival time / Tiempo de sobrevida ______ min.
Survival time = ($\log_{10}$ labeled population - 2) x labeled D-value

Kill time / Tiempo de muerte ______ min.
Kill time = ($\log_{10}$ labeled population + 4) x labeled D-value

D - value / Valor D (132 °C) ______ sec./seg.

Survival time / Tiempo de sobrevida ______ min.
Survival time = ($\log_{10}$ labeled population - 2) x labeled D-value

Kill time / Tiempo de muerte ______ min.
Kill time = ($\log_{10}$ labeled population + 4) x labeled D-value

D - value / Valor D (135 °C) ______ sec./seg.

Survival time / Tiempo de sobrevida ______ sec./seg.
Survival time = ($\log_{10}$ labeled population - 2) x labeled D-value

Kill time / Tiempo de muerte ______ min.
Kill time = ($\log_{10}$ labeled population + 4) x labeled D-value

Z-value / Valor Z ______ °C

Parameters determined at time of manufacture according to ISO 11138-1: 2017, ISO 11138-3: 2017 and IRAM 37102: 1999 (Parts 1 and 3) standards. The values shown are reproducible only under the same conditions under which they were determined.

Parámetros determinados al momento de la fabricación según normas ISO 11138-1: 2017, ISO 11138-3: 2017 e IRAM 37102: 1999 (Partes 1 y 3). Los valores presentados son reproducibles solo bajo las mismas condiciones en las cuales fueron determinados.

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Lic. Adrián J. Rovetto
Director Técnico
Technical Director

Therefore, each BI batch is accompanied by a COA that includes an evaluation of key parameters (D-Value, KT, ST) at three different temperatures, which can also be used to determine the Z-Value. Moreover, the COA provides information about the spore population in the biological indicator.

In order to better understand the application of these parameters, it is first essential to define them:

Population

The nominal population refers to the number of viable microorganisms contained in the BI, and it must be stated by the manufacturer in the COA. This value is expressed in colony-forming units (CFU), representing visible colonies that originate from one or more microbial cells capable of growth. The population is considered a resistance parameter as it is essential for calculating the D-value and for determining both the KT and ST.
If a user needs to independently verify the viable spore population, ISO 11138-1 specifies that the result must fall within 50% to 300% of the nominal population declared by the manufacturer to be considered acceptable. Furthermore, according to the same standard, the manufacturer must provide, upon request, the internal protocol used to determine the initial viable population, which must be followed thoroughly by the user.

Terragene provides its protocol in the Technical Note #04: Spore Count Protocol, which is based on Annex A of ISO 11138-1, to ensure reliability and regulatory compliance. This document includes specifications for the culture medium as well as all necessary steps to support accurate and standardized population verification by end users.

D-value

The D-value represents the time—in the case of gamma radiation, the dose—required to reduce the microbial population by 90%, thus meaning a reduction of 1 logarithmic unit. For example, a BI with an initial population of 1×10^6 (1 000 000) spores and a D-Value of 2.2 minutes means that it would take 2.2 minutes to reduce the spore population to 100 000 under the specified conditions. The D-Value is a crucial parameter for verifying that the sterilization process achieves the desired level of microbial reduction.

Annexes C and D of ISO 11138 list several methods for calculating the D-Value. Among these, Terragene uses the limited Holcomb-Spearman-Karber (LHSKP) method. This method has the advantage of using a time variable that increases in equal intervals for each test.

Kill time

The kill time (KT) is the minimum time required to reduce the microbial population to an acceptable level under specific conditions. The KT is a measure of microbial resistance: after this time, the population will be significantly reduced.

The formula to calculate the KT is:

$$KT = (\log_{10} \text{population} + 4) \times (\text{D-value})$$

The term "+4" in the equation adds four additional logarithmic reductions beyond the level required to reduce the spore population to an acceptable threshold. This ensures that all spores are effectively eliminated during the sterilization process.

Survival time

The survival time (ST) is the maximum period during which most microorganisms remain aliveunder the specified conditions. This parameter helps estimate the maximum exposure time that does not result in the death of microorganisms, meaning the population remains viable throughout the entire sterilization cycle.

The ST can be calculated using the D-Value:

$$ST = (\log_{10} \text{population} - 2) \times (\text{D-value})$$

The term "-2" takes into account the initial logarithmic reductions required before reaching a level where only 100 viable spores remain. The ST is useful for determining the maximum time needed in the sterilization cycle before spores begin to die.

Z-value

The Z-value is defined as the change in the exposure temperature that corresponds to a tenfold (one-log) change in the D-Value. In other words, the Z-value provides insight into how sensitive the biological indicator (BI) is to different temperatures: when comparing two BI batches with different Z-Values, the batch with the lower Z-value will be more sensitive to temperature changes than the other.

Outlined below are the minimum values established by the standards:

ISO 11138	ISO 11138-2 EO	ISO 11138-3 STEAM	ISO 11138-4 DRY HEAT	ISO 11138-5 FORM
Population (CFU/vial)	$\geq 1.0 \times 10^6$	$\geq 1.0 \times 10^5$	$\geq 1.0 \times 10^6$	$\geq 1.0 \times 10^5$
D-value (min)	mix EO $\mathbf{D_{54}} \geq 2.5$ 100% EO $\mathbf{D_{54}} \geq 2$	$D_{121} \geq 1.5$	$D_{160} \geq 2$	$D_{60} \geq 6$
Z-value (°C)	NA	$Z \geq 6$ (110 °C-138 °C)	$Z \geq 20$ (150 °C-180 °C)	NA
Kill time (min)*	mix EO $\mathbf{KT_{54}} \leq 25$ 100% EO $\mathbf{KT_{54}} \leq 20$	$KT_{121} \leq 13.5$	$KT_{160} \leq 20$	$KT_{60} \leq 54$
Survival time (min)*	mix EO $\mathbf{ST_{54}} \geq 10$ 100% EO $\mathbf{ST_{54}} \geq 10$	$ST_{121} \geq 4.5$	$ST_{160} \geq 8$	$ST_{60} \geq 18$

*KT and ST specified with the **minimum population** and **minimum D-value**

The Importance of the D-value

Understanding the resistance of the BIs allows us to select the most suitable batch for cycles that are less demanding than the standard ones.

If routine monitoring of highly demanding cycles is required, it is recommended to use a BI batch with resistance close to the minimum value established by the standard. Conversely, if the goal is to assess the lethality of more robust or higher-intensity processes, batches with a higher D-value should be selected.

It is important to note that these parameters are determined using specialized equipment, such as resistometers or laboratory systems. Unlike these instruments, conventional sterilizers incorporate multiple stages within a standard sterilization cycle that contribute to the overall lethality of the process, making resistance parameters even more conservative.

The importance of the Z-value

In certain industries, such as the pharmaceutical and food industries, materials are often sterilized at non-standard temperatures, meaning temperatures other than 121, 132, or 135°C. Therefore, the Z-value is particularly useful for determining how a BI would behave when exposed to a temperature not reported in the Certificate of Analysis (COA).

From a graphical perspective, the Z-value is the negative reciprocal of the slope of the line connecting two points on the semi-logarithmic plot of D-value vs. temperature. Using the provided Z-value, the target working temperature, and a known D-value (established at a different temperature), the D-value for a specific cycle can thus be recalculated.

For instance, to determine the D-value of a BI at a second temperature (T2), the following equation must be used:

$$\log \boldsymbol{D_2} = \log D_1 - \frac{(T_2 - T_1)}{z}$$

Where:

*D*1: is the D-value at *T*1 (informed in the CoA)
*D*2: is the D-value at *T*2 (the working temperature)
*T*1: is the reference temperature (informed in the CoA)
*T*2: is the working temperature
z: is the Z-value (informed in the CoA).

Rearranging the equation:

$$\boldsymbol{D_2} = 10\hat{}\left(-\frac{(T_2 - T_1)}{z} + \log D_1\right)$$

It is important to bear in mind that it is first necessary to validate with the BI manufacturer the linearity range for which this equation can be applied.

Conclusion

The correct interpretation and application of the resistance parameters of a BI are essential for optimizing sterilization cycles and ensuring product reliability. Understanding the BI characteristics is as important as understanding the sterilization process and the material to be processed in order to ensure a safe and effective cycle.

References

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