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Calculation of the Biological Indicator D-value and Kill Time for a Temperature not Specified on the Certificate of Analysis

Data presented on the Certificate of Analysis (C of A) for biological indicators (BI) used to monitor steam sterilization processes typically include the spore population, D-value(s) determined at one or more temperatures and the Z-value.

Often, the end users of the BIs are operating their sterilizers at a temperature for which the D-value is not reported on the C of A. A common question BI users ask is "how can I calculate the D-value and kill time of the BI when my sterilization temperature is not one of those listed on the Certificate of Analysis?"

To calculate the theoretical kill time of the BI at a specified temperature, two data points are required; the initial population of the BI, and the D-value at the specified temperature. This Spore News will address the methods for calculating the D-value and the theoretical kill time in these situations. For the purpose of this discussion, we will not be using the survival/kill time calculation formulas as presented in ISO and USP[1]. These formulas add a safety factor into the calculations resulting in values that generally bracket the empirically derived data. In other words, the calculated kill time value will be higher than the empirically determined kill time.

Let's begin by calculating the theoretical kill time for a temperature that **is** listed on the C of A. Data extracted from a C of A for a BI lot (Figure 1) shall be used in the following examples.

[1]Survival time/dose = not less than the D-value X ($\log_{10}$ labeled viable test organism count per carrier – 2)
Kill time/dose = not more than the D-value X ($\log_{10}$ labeled viable test organism count per carrier + 4)

Lot No.: S-308

Heat Shocked Population: 2.6×10^6 Spores/Unit

Assayed Resistance:

Temperature	D-value[1]	Survival	Kill	
121ºC	1.7	7.51[2]	17.71 [2]	minutes
132ºC	0.3	1.0[3]	3.0 [3]	minutes
134ºC	0.3	1.0[3]	2.5 [3]	minutes
135ºC	0.2	0.5[3]	2.0 [3]	minutes

Z-value 15.2 °C

D-value reproducible only when exposed in an AAMI BIER vessel and cultured under the exact conditions used to obtain results reported here. MPN and Survivor Curve method used.

Units are manufactured in compliance with Mesa's quality standards, USP, EN 866 and ISO 11138 guidelines and all appropriate subsections.

[1] D-value calculated using the Limited Holcomb-Spearman-Karber method.

[2] Survival/Kill values are calculated according to USP and ISO 11138.

[3] Empirically derived data.

Figure 1. Certificate of Analysis for BI lot S-308

EXAMPLE 1. Calculation of the BI Theoretical Kill Time at a Sterilization Temperature of 121ºC

The theoretical kill time is a product of the log of the population (N_0) plus one (1) and the D-value.

Theoretical kill time = (Log_{10} N_0 + 1) x D-value

The first step of this equation is to take the log of the initial population. It is often assumed that this will be the spore log reduction (SLR) required to reduce the spore population to zero surviving spores. This in fact is not true. This SLR will reduce the spore population to an average of 10^0 or one ($10^0 = 1$) surviving spore per BI (see Figure 2). One is added to the SLR in the calculation to reduce the number of spores to less than one per unit resulting in total kill of all test units.

From the C of A for lot S-308 (Figure 1), the Heat Shocked population (N_0) is 2.6×10^6 per unit and the D_{121}-value is 1.7 minutes. Substituting these values into the equation we have:

$$\text{Theoretical kill time} = (\text{Log}_{10}\ 2.6 \times 10^6 + 1) \times 1.7 \text{ minutes} = 12.6 \text{ minutes}$$

A single BI should be killed in approximately 12.6 minutes. During data collection on BIs from lot S-308, the 12 minute exposure at 121ºC resulted in 8 units killed out of 20 exposed. Tracking the total spores of this exposure we have 2.6 x 10^6 spores per unit multiplied by 20 units resulting in 5.2 x 10^7 spores. The theoretical kill time would then be (Log_{10} 5.2 x 10^7 + 1) x 1.7 minutes = 14.8 minutes. The empirical data from the 14 minute exposures resulted in 20 units killed out of 20 exposed. So we see that the empirical data closely matches the theoretical calculations.

Time (min)	Population per unit	Population per 20 units
0	2.6 X 10^6	5.2 X 10^7
1.7	2.6 X 10^5	5.2 X 10^6
3.4	2.6 X 10^4	5.2 X 10^5
5.1	2.6 X 10^3	5.2 X 10^4
6.8	2.6 X 10^2	5.2 X 10^3
8.5	2.6 X 10^1	5.2 X 10^2
10.2	2.6 X 10^0	5.2 X 10^1
11.9	2.6 X 10^{-1}	5.2 X 10^0
13.6	2.6 X 10^{-2}	5.2 X 10^{-1}

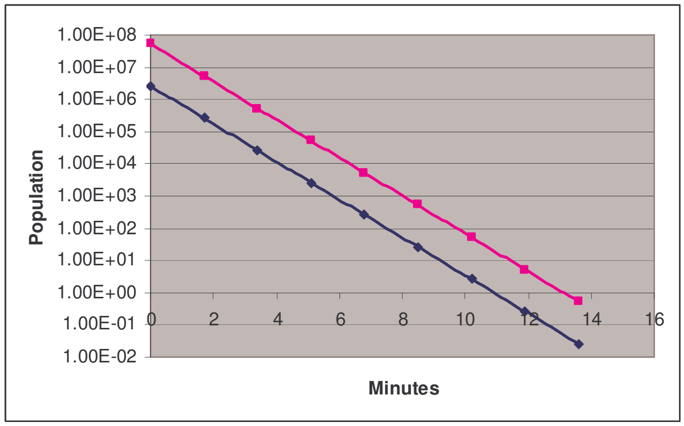

Figure 2. An example of a Spore Log Reduction (SLR)

EXAMPLE 2. Calculation of the BI D-value and Theoretical Kill Time at a Sterilization Temperature of 131ºC

To calculate the theoretical kill time of a BI at a temperature not listed on the C of A, the Z-value must be considered. According to ISO 11138, the Z-value is defined as "…the change in exposure temperature which corresponds to a 10-fold change in D-value." In a practical sense, it is a measure of how susceptible a spore population is to changes in temperature. For example, if the Z-value is 10ºC, then increasing the sterilization temperature by 10ºC degrees will result in a one log reduction of the D-value. If the Z-value of lot S-308 were 10ºC rather than 15.2ºC, the D_{131}-value would simply be a one log difference from the D_{121}-value of 1.7 minutes or 0.17 minutes. Rarely does the situation occur where the mathematical calculations are this simple.

To determine the kill time for lot S-308, the first step will be to calculate the D_{131}-value using the correct Z-value. When the Z-value and the actual sterilization temperature are not convenient numbers to work with mathematically, the following formula (derived from the Z-value calculation formula presented in ISO 11138-3:1995(E) Annex B is necessary:

$$D_1 = D_2 \times 10^{\frac{T_2 - T_1}{Z}}$$

Where: D_1 is the D-value at temperature T_1 (e.g. the D_{131}-value)
D_2 is the known D-value at temperature T_2 (e.g. D_{121}-value = 1.7 minutes)
Z is the Z-value (e.g. 15.2ºC)

Inserting these values into the formula we have:

$$D_{131} = 1.7 \times 10^{\frac{121-131}{15.2}}$$

$$D_{131} = 0.4 \text{ minutes}$$

Theoretical kill time for the BI at 131ºC = (Log_{10} 2.6 x 10^6 + 1) x 0.4 minutes = 3.0 minutes.

EXAMPLE 3 Calculation of the BI D-value and Theoretical Kill Time at a Sterilization Temperature of 116.5ºC

To determine the kill time, the first step will be to calculate the $D_{116.5}$-value using the formula:

$$D_1 = D_2 \times 10^{\frac{T_2 - T_1}{Z}}$$

Where: D_1 is the D-value at temperature T_1 (e.g. the $D_{116.5}$-value)
D_2 is the known D-value at temperature T_2 (e.g. D_{121}-value = 1.7 minutes)
Z is the Z-value (e.g. 15.2ºC)

Inserting these values into the formula we have:

$$D_{116.5} = 1.7 \times 10^{\frac{121-116.5}{15.2}}$$

$$D_{116.5} = 3.4 \text{ minutes}$$

Theoretical kill time for the BI at 116.5ºC = (Log_{10} 2.6 x 10^6 + 1) x 3.4 minutes = 25.2 minutes.

<u>EXAMPLE 4 Calculation of the BI D-value and Theoretical Kill Time at a Sterilization Temperature of 124ºC</u>

To determine the kill time, the first step will be to calculate the D_{124}-value using the formula:

$$D_1 = D_2 \times 10^{\frac{T_2 - T_1}{Z}}$$

Where: D_1 is the D-value at temperature T_1 (e.g. the D_{124}-value)
D_2 is the known D-value at temperature T_2 (e.g. D_{121}-value = 1.7 minutes)
Z is the Z-value (e.g. 15.2ºC)

Inserting these values into the formula we have:

$$D_{124} = 1.7 \times 10^{\frac{121-124}{15.2}}$$

$$D_{124} = 1.1 \text{ minutes}$$

Theoretical kill time for the BI at 124ºC = (Log_{10} 2.6 x 10^6 +1) x 1.1 minutes = 8.2 minutes.

A single BI should be killed in approximately 8.2 minutes. During data collection on BIs from lot S-308, the 8 minute exposure at 124ºC resulted in 17 units killed out of 20 exposed.

The D_{124}-value was one of the D-values experimentally determined on lot S-308 and used in the Z-value calculation. The D_{124}-value was determined to be 1.0 minute and the 9 minute exposure resulted in total kill of all 20 BIs.

The calculations very closely match the experimentally determined data.

Discussion

Z-value curves for *Geobacillus stearothermophilus* spores tend to be concave downward over a wide temperature range (i.e. the Z-values tend to decrease as the temperature increases). The fact that resistometers (test vessels) perform most accurately at temperatures below 130ºC certainly contributes to this situation.

For the BI manufacturer, ISO states that a Z-value shall be calculated from D-values obtained in the temperature range of 110ºC-130ºC. Mesa calculates the Z-value using D-values obtained from three temperatures in the 120ºC-130ºC range.

Based on the ISO requirements and Mesa current operating procedure for Z-value determinations, this formula will result in a good estimation of D-values in the 110ºC-130ºC temperature range using the Z-value printed on the C of A . This is supported by comparing the empirically determined results with the calculated results as in examples 1 and 4.

Please email us with topics you would like to see addressed in "Spore News".