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Garrett Krushefski
Scientific & Technical Services Manager

The Quest for the Perfect D-value

Here's a quick quiz!

Which of the following two steam biological indicators would be more difficult to kill?
Lot A has 2.0 X 10^5 spores per indicator, D_{121}-value = 2.2-minutes,
Lot B has 3.3 X 10^6 spores per indicator, D_{121}-value = 1.8-minutes.

Lot A has a slightly greater D-value but Lot B has more than ten times as many spores. So, which is more difficult to kill? Not sure? Keep reading and we'll figure it out.

There are two aspects that together determine the overall resistance capabilities of a biological indicator: population and D-value. This edition of Spore News will evaluate these two aspects as well as discuss how specific one really needs to be when ordering a new lot of indicators.

SQUARE WAVE LETHALITY

D-value is defined as the time at a specified set of exposure conditions to reduce the viable spore population by one-log (or 90%). D-value assessments are conducted by exposing biological indicators in a resistometer. ISO-18472:2006 addresses the performance standards and requirements that must be met for biological and chemical indicator test equipment while ISO-11138:2006 discusses the set points to be used when testing biological indicator D-values. To summarize the steam resistometer cycle requirements, the vessel must be able to:

- Evacuate the chamber to 0.65 psia in 120 seconds or less without the aid of a steam flush,
- Charge to within -0.5ºC of the exposure temperature set-point within 10 seconds or less,
- Maintain exposure temperature (+/- 0.5ºC) and corresponding saturated steam pressure (+/- 0.5 psia) during the exposure phase,
- At exposure completion, evacuate the chamber to non-lethal conditions to attain 100ºC within 5 seconds and to a pressure of 1.4 psia within 60 seconds.

The precise cycle performance characteristics of a resistometer deliver what is referred to as "square-wave lethality". The ability to deliver exact and distinct "doses" of lethality allows us to see slight differences in resistance performance from lot to lot. To illustrate these subtle differences, review the fraction-negative data sets for four lots of EZTest steam biological indicators presented in Table 1. The values are presented as number of negative units/number of units exposed with empirical kill times* highlighted.

Table 1. Comparison of EZTest Steam Fraction-negative D-value Data

BIER Cycle Exposure Time (minutes)	Lot # and Spore Population per Unit			
	Lot A 2.0×10^5	Lot B 3.3×10^6	Lot C 2.5×10^6	Lot D 1.9×10^6
09	00/20	00/25	00/25	---
10	00/20	00/25	00/25	00/25
11	00/20	00/25	07/25	00/25
12	05/20	11/25	12/25	00/25
13	18/20	17/25	17/25	03/25
14	19/20	24/25	25/25	10/25
15	20/20	25/25	25/25	13/25
16	20/20	25/25	25/25	20/25
17	20/20	25/25	---	23/25
18	---	---	---	25/25
19	---	---	---	25/25
LHSK D-value	2.2336	1.8348	1.8311	2.2681
Rounded LHSK D-value	2.2	1.8	1.8	2.3

* The empirical kill time is defined as the *shortest* BIER exposure at which all units were negative for growth *and* at all exposures of longer duration, no growth was observed.
** The empirical survival time is defined as the *longest* BIER exposure at which all units were positive for growth *and* at all exposures of shorter duration, 100% growth was observed.

These subtle differences in fraction-negative data are detectable only in a resistometer which delivers square-wave lethality. And what about our quiz? According to the fraction-negative data set, Lot A and Lot B have an identical 15-minute empirical kill time. Further observation shows that the empirical survival time** (11.0-minutes) is also the same for both lots.

There is a simple formula that can help one to have predicted these results. It is referred to as F_{BIO} value[1] and is calculated as follows:

$F_{BIO} = \text{Log}_{10}$ population X D-value

[1] ISO 11138-1:2006

The F_{BIO} for Lot A is 11.6622
Log_{10} 2.0 X 10^5 = 5.3010
5.3010 X 2.2 = 11.6622

The F_{BIO} for Lot B is 11.7333
Log_{10} 3.3 X 10^6 = 6.5185
6.5185 X 1.8 = 11.7333

For comparison, the F_{BIO} for Lot C is 11.5162 and for Lot D it is 14.4412.
It is with increasing frequency that we receive requests for biological indicators (BIs) with a very specific and narrow range of acceptable D-values and/or spore population. As an example, a recent caller requested an EZTest steam indicator with population less than or equal to 2.0 X 10^6 spores per unit. The available lot had a label claim of 2.2 X 10^6. This was deemed "unacceptable" by the client as they were operating under a perception that an indicator with more than 2.0 X 10^6 spores per unit would have too great of a potential to survive their sterilization process. In this particular case, any D-value would have been acceptable, as long as the population per unit did not exceed 2.0 X 10^6. Had Lot D been available, the client would have accepted delivery. Notice how this lot actually poses the greatest challenge to the sterilization system as it has the longest empirical kill time and the greatest F_{BIO}.

PROCESS VESSEL LETHALITY
When purchasing a BI for monitoring a validated steam cycle, how specific does one need to be in specifying population and D-value requirements? To answer this question, we must consider the difference in resistometer performance compared to that of a production steam autoclave. A resistometer delivers square-wave lethality to the biological indicator. By comparison, a process vessel operates with much more variation in conditions. The time for the chamber to reach the exposure set-point may take many minutes in a steam autoclave whereas the BIER will achieve exposure conditions within 10 seconds. Temperature during the exposure phase may fluctuate by several degrees in a process vessel and the autoclave will take substantially longer than 5 seconds to reduce the chamber temperature to values less than 100ºC. The result is delivery of lethality that is VERY different than the square-wave BIER performance. However, the spores respond to these dynamics in a very predictable manner. As a result of the dynamic conditions that exist in a process vessel, it is virtually impossible to differentiate between the subtle differences in lot-to-lot resistance performance such as those seen in Table 1.

THE FUNCTION OF THE BIOLOGICAL INDICATOR AS A MONITOR OF CYCLE PERFORMANCE
The BI as a routine monitor of a validated process is a system to be used to detect catastrophic process failures. Currently, no physical instrumentation systems are available that can be embedded in the most difficult to sterilize location of the product to measure all critical process parameters. The bacterial spores in the BI effectively integrate all critical process parameters at all times during the cycle and respond to the exact lethal conditions that exist in the specific location in which the BI is placed.

CONCLUSION

The standards for steam BIER vessels require the most accurate and responsive instruments available. Tight performance parameters result in delivery of square-wave lethality to the exposed BIs. The ability to deliver this type of steam lethality allows one to observe the slight differences seen in Table 1. The dynamic conditions within a process vessel are highly dependent on the characteristics of the load and in this type of environment, it is impossible to ascertain these subtle differences in resistance performance.

The unexpected positive test unit in a validated process is not due to use of a BI with D-value or spore population that is slightly greater than the desired level. The positive BI in a validated cycle results from a loss of a substantial amount of lethality.

The D-value on the Certificate of Analysis is an **accurate estimate** of resistance for a **specific set of exposure conditions**. It can have an effect during cycle development but is not a concern for positive BIs in a validated, full cycle production run.

To completely illustrate this point, one must consider the remainder of the story…

- We agree that the D-value is the time that it takes at a **specific set of conditions** to reduce the population of spores in the BI by one-log or 90%.
- We also agree that process vessels, because of the load, will operate at different conditions than BIER vessels.

How does one apply the BIER vessel data to the process? This is accomplished through the application of the Z-value. The Z-value is defined as the temperature change required to change the D-value by one-log or 90%. The Z-value provides us a means of integrating process lethality delivered at conditions other than that delivered by the BIER during D-value assessment. The Z-value is used to establish an equivalent lethality per unit of process clock time. This allows total delivered lethality to be expressed as Spore Log Reductions (SLRs) which are then used to calculate Sterility Assurance Level (SAL). We'll continue discussion of this topic in a future Spore News.

The first step in our journey to establishing a SAL is the D-value but it is only the *first step*. We need to look beyond the D-value to uncover the rest of the SAL story.

For additional discussion on Z-value, please read Spore News Volume 3, Number 1 and Volume 3, Number 2. To access these and all other Spore News, click on the Spore News link from our home page www.sgmbiotech.com .

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