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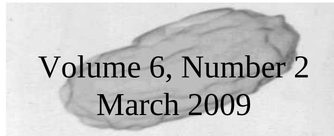

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The Biological Indicator Spore Population has Minimal Impact on Resistance Performance

SGM receives many requests from customers for very specific spore populations on the BIs. These requests, in many cases, are a result of purchase specification requirements established by well meaning individuals. Requirements that significantly influence product performance qualities are essential. Some purchase specification requirements simply make the product more expensive, may delay orders or create other issues for both the manufacturer as well as the user. Many times these requirements don't provide the user with the product qualities really needed which is consistent performance in monitoring the sterilization process. SGM makes every attempt to honor these requests and our customers' needs. We recently had to delay a customer's order because a population assay was 2.1×10^6 and the purchase specification required a population between 1.5×10^6 and 2.0×10^6. The customer had to wait three weeks for a new lot to be made and SGM incurred all associated manufacturing fees to produce a lot that would meet the purchase specification. As you will see, neither of these inconveniences (delay or cost) was necessary.

The point of this Spore News is "prepare your purchase specifications based on sound science" and not an emotional feel good value. First, let us consider the spore population label claim verification testing. USP[1] and ISO state that recovery values within 50% to 300% of the label claim are acceptable. This may seem like a HUGE range until one considers the overall impact this would have on the resistance performance for the indicator. It is relatively easy to mentally calculate the lower limit (LL) and upper limit (UL) using the ISO method. For that reason, the calculation using the USP method (see footnote) follows:

LL = $(\text{Log}_{10}\ 2.0 \text{ X } 10^6) - 0.3$

LL = 6.3 - 0.3 = 6.0; converted back to scientific notation,

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[1] USP 32 reads, "The requirements of the test are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48." Mathematically, this is equivalent to the ISO requirement of 50% to 300%.

Inverse Log of 6.0 = 1.0×10^6

UL = (Log_{10} 2.0×10^6) + 0.48

UL = 6.3 + 0.48 = 6.78; converted back to scientific notation,

Inverse Log of 6.48 = 6.0×10^6

Again, the difference between 1 million and 6 million spores seems quite large. But as we will soon see, the difference is quite insignificant.

Table 1 outlines the spore death kinetics for three theoretical BI lots with spore populations of 1 million, 2 million and 6 million spores per BI. Each lot has a 4.0 minute D_{EO}-value.

Table 1. Three lots with identical D-value

	Lot A 1.0×10^6 spores per strip		Lot B 2.0×10^6 spores per strip		Lot C 6.0×10^6 spores per strip	
Exposure Time	**Number of surviving spores per strip**	**# of positive strips Per 100 exposed**	**Number of surviving spores per strip**	**# of positive strips Per 100 exposed**	**Number of surviving spores per strip**	**# of positive strips Per 100 exposed**
0	1.0×10^6	100	2.0×10^6	100	6.0×10^6	100
4	1.0×10^5	100	2.0×10^5	100	6.0×10^5	100
8	1.0×10^4	100	2.0×10^4	100	6.0×10^4	100
12	1.0×10^3	100	2.0×10^3	100	6.0×10^3	100
16	1.0×10^2	100	2.0×10^2	100	6.0×10^2	100
20	1.0×10^1	100	2.0×10^1	100	6.0×10^1	100
24	1.0×10^0	63	2.0×10^0	86	6.0×10^0	100
28	1.0×10^{-1}	10	2.0×10^{-1}	18	6.0×10^{-1}	45
32	1.0×10^{-2}	1	2.0×10^{-2}	2	6.0×10^{-2}	6
36	1.0×10^{-3}	0	2.0×10^{-3}	0	6.0×10^{-3}	0
40	1.0×10^{-4}	0	2.0×10^{-4}	0	6.0×10^{-4}	0

Note that despite the number of spores present on each strip, all 100 test strips from each of the three lots would be killed in the 36.0 minute exposure. At all shorter exposures, positives would be observed. From a user standpoint, any of the three lots could be used to monitor a sterilization cycle and virtually no difference would be detected. The subtle differences in the number of positive units as shown in this table could only be detected in a BIER (biological indicator evaluator resistometer) which is designed to deliver tightly-controlled, distinct “doses” of lethality.

While zero positives are expected for any of the lots when exposing 100 strips in the 36 minute cycle, we can calculate the percent chance for survival using the following equation.

$$\text{\# of negative units per 100 exposed} = \frac{100}{\text{inverse natural log (\# of surviving spores per BI)}}$$

Thus, for Lot B the chance for a BI to test positive from the 36 minute exposure is:

$$\text{\# of negative units per 100 exposed} = \frac{100}{\text{Inv ln } (2.0 \text{ X } 10^{-3})}$$

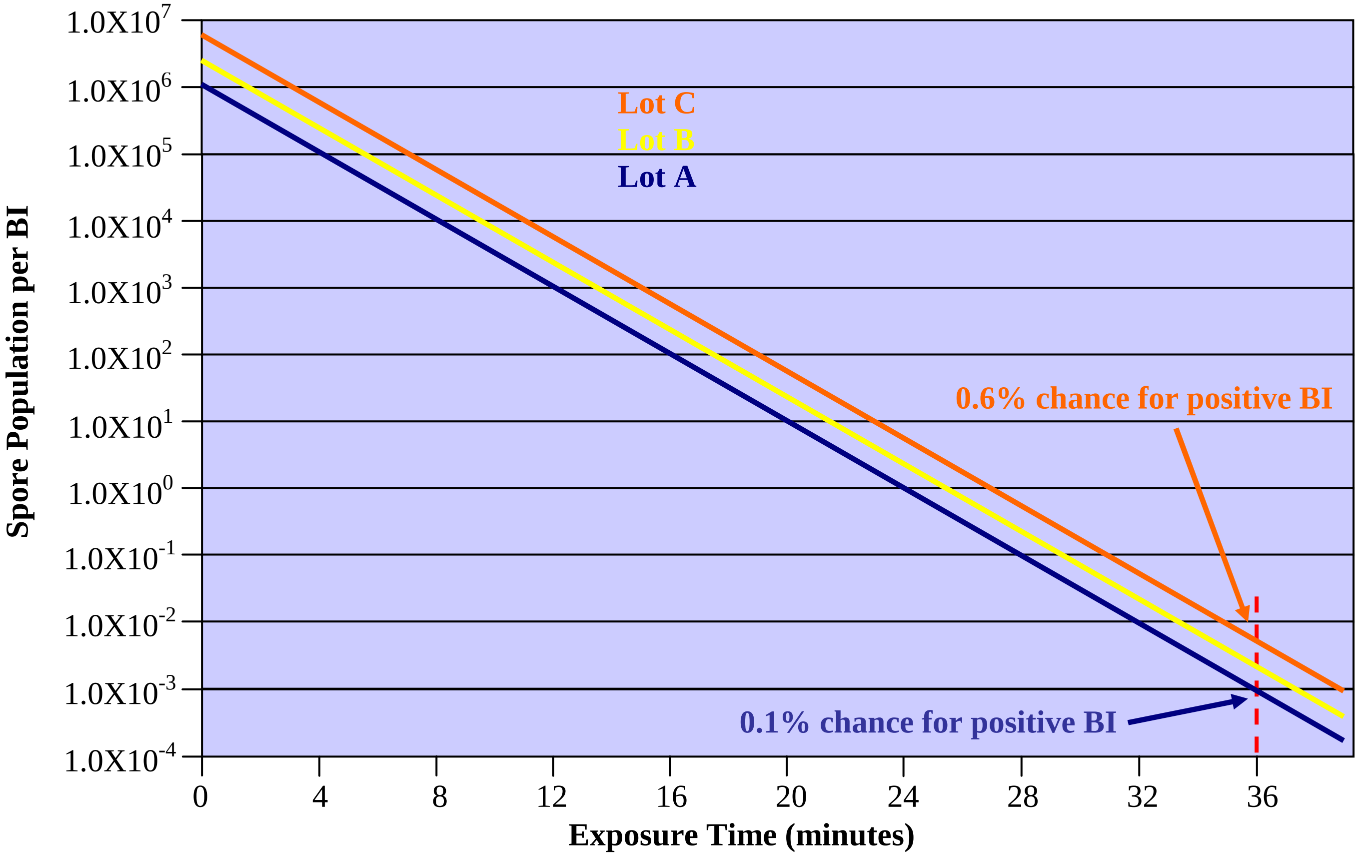
$$\text{\# of negative units per 100 exposed} = \frac{100}{1.00200} = 99.8$$

Thus, one will have 99.8 negative units per 100 exposed; or a 0.2 % chance for any individual strip from Lot B to test positive after having been exposed to the 36 minute exposure.

For Lot A, there is a 0.1% chance for a positive BI from the 36 minute exposure.
For Lot C, there is a 0.6% chance for a positive BI from the 36 minute exposure.

We now see how the seemingly large difference in 1 million or 6 million spores per BI is reduced to an insignificant difference in potential for survival.

The following is a graphical representation of the relevant spore death kinetics.



All of the above discussion was based upon theoretical BIs. Let us now look at three actual lots to see how difficult they are to kill.

The spore population for BATR-352 is 2.8 X 10^6, D_{EO}-value = 4.2 minutes.
The spore population for BATR-361 is 3.3 X 10^6, D_{EO}-value = 3.8 minutes.
The spore population for BATR-365 is 8.3 X 10^6, D_{EO}-value = 3.4 minutes.

So, which lot will be the easiest to kill? The answer may surprise you, although well read Spore News readers will have an advantage in correctly answering that question (i.e. see Spore News Volume 4, Number 3, May 2007).

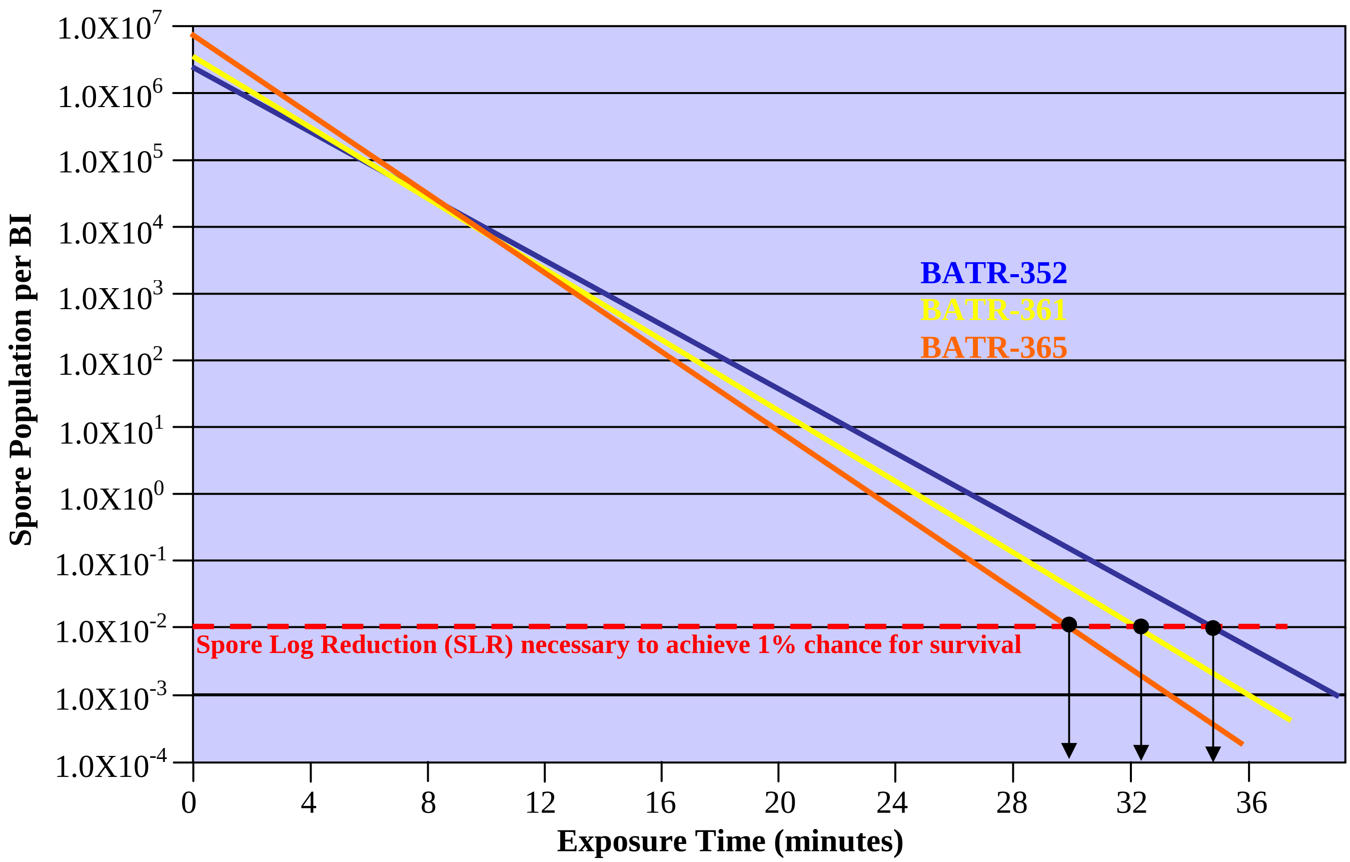
The answer… *BATR-365 with its 8.3-million spores is the <u>easiest</u> to kill!*

Table 2 presents the resistance performance data that appears on the Certificate of Analysis for each lot.

Table 2. Comparison of Resistance Performance for Three Lots

Lot #	BATR-352	BATR-361	BATR-365
Population Per Strip	2.8 X 10^6	3.3 X 10^6	8.3 X 10^6
Ethylene Oxide D-value	4.2	3.8	3.4
Calculated Survival Time	18.62	16.95	16.66
Calculated Kill Time	43.74	39.47	36.98
Dry Heat $D_{160°C}$-value	2.2	3.3	1.6
Calculated Survival Time	9.60	15.02	7.86
Calculated Kill Time	22.55	34.96	17.45
	(minutes)		

The graph below presents the log-linear death curve for each lot from Table 2. Examination of the graph helps to illustrate how it is possible that a strip with more than twice as many spores as either of the other two will be inactivated first.



The purchase specification is intended to secure a product that will provide you with a consistent challenge for monitoring your sterilization process. Performance is the key and spore population is one element that influences it, **but only one**. Similar to a jigsaw puzzle, you can not see the whole picture by focusing on one piece. Spore population on the BI is only one piece of the sterilization puzzle.

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