SporeNews
biological indicators newsletter
MesaLabs

Volume 9, No. 4

Using replicate BIs to evaluate biodecontamination cycles in isolators

by Garrett Krushefski

If you are involved with the world of sterilization you are no doubt familiar with the "unexpected positive biological indicator" and the subsequent problems that ensue. If your involvement in sterilization is related to vapor phase hydrogen peroxide (VHP) at ambient pressures, then this subject can lead to sleepless nights.

Other sterilization processes, such as saturated steam under pressure with a pre-vacuum air removal, have penetrative capability. The same is not true for ambient pressure gassing processes. Due to the limited penetrating capability of VHP, presentation of the spores on the carrier surface is critical. In addition, any presence of debris amongst the spores can also lead to spores surviving VHP exposures where kill was otherwise expected; a concept referred to as "rogue BIs" in various publications. [1,2]

How then does one proceed should the undesirable, unexpected growth-positive BI appear during cycle qualification and/or requalification studies? Once the BI has been cultured into broth and the growth-positive result is obtained, it is impossible to determine if the growth was due to a flaw in the presentation of the spores or if it was due to a deviation in expected cycle lethality. When only one BI is used at each test location, the unfortunate answer to this "what now?" question often involves costly retest cycles. Section 8.1 of PDA Technical Report No. 51 suggests that re-running the cycle with a different lot of BIs or re-running the cycle with duplicate or triplicate BIs in the same location may be necessary.[3]

This edition of Spore News will discuss the concept of using triplicate BIs, not as a reaction to having obtained an unexpected growth-positive BI in your VHP cycle, but as a proactive measure. In the previously cited publication, James Drinkwater references a case study that suggested even the highest quality inoculated stainless steel carriers will have a failure rate on the order of 0.3%. Thus, if the rogue BI is an item with which we must learn to live, why not be prepared for the inevitable, albeit undesirable situation?

The reason to use multiple BIs at each test location is because we need each BI to be a statistical replicate of its neighbor. I understand there may be reader skepticism at this point as we have a manufacturer of BIs endorsing a policy that seemingly increases BI consumption three-fold. However, this does not need to be the case. Instead of monitoring 100 discrete locations throughout the isolator, the validation technician can identify the 30 or 40 most difficult to sterilize locations and use triplicate BIs at each of these challenge locations, thus consuming 90 or 120 BIs per cycle. With statistical replicates in place, we now have the ability to use the Halvorson-Ziegler equation[4] to calculate the most probable number (MPN) of surviving organisms IF we observe a case where one (or two) of the three replicate BIs test growth-positive. When only one BI is used at each test location, and that one BI yields a growth-positive result, there is no way to calculate if that result was due to one surviving spore, or hundreds-of-thousands of surviving spores. The Halvorson-Ziegler equation is:

$$MPN = \ln(n/r)$$

where:
MPN = Most Probable Number of surviving spores
ln = natural log function
n = number of replicate BIs at each discrete test location
r = number of growth-negative BIs at each discrete test location

Let us look at an example of how we would proceed if in a VHP cycle we observed one positive and two negative BIs (i.e. n = 3 and r = 2) at a particular location where triplicate BIs were used.

MPN = ln (3/2) = 0.405

What this MPN number indicates is that on average we have 0.405 surviving spores per BI. Two of the three BIs were growth-negative and thus we know that there were zero surviving spores on each of those two BIs. Now that we've calculated the MPN value, the next step is to use this number to calculate the spore log reduction (SLR) associated with this observation of two negative BIs and one positive BI. For this we have the following equation:

$SLR = Log_{10} No - Log_{10} MPN$

where:
SLR = spore log reduction
No = the initial spore population on the non-exposed BI

If the Certificate of Analysis for the lot of BIs used indicated an initial spore population of 1.6×10^6 spores per BI, the SLR calculation would be:

$SLR = Log_{10} 1.6 \times 10^6 - Log_{10} 0.405$
$SLR = 6.204 - (-0.393)$
$SLR = 6.597$

Thus, one can see that despite the one growth-positive BI at the location of the triplicate BIs, one can still document that a 6+ spore log reduction was achieved at that particular test location. Do be advised that this calculation is ONLY possible when replicate BIs are used. If one were to distribute 100 BIs at 100 discrete test locations, it would not be appropriate to perform the above MPN & SLR calculation as these 100 individual BIs are not replicates of the others.

The next question one might be tempted to ask is, *Was that growth-positive BI due to an imperfection in the presentation of the spores…or was it due to a slight deviation in cycle lethality at that particular test location?* Without intending to sound flippant, I suggest the answer to that particular question is, *Does it matter?* After all, we do have growth-negative observations at that location and the mathematical analysis demonstrates that a 6+ SLR was achieved. Granted, if all three of the replicates are growth-positive, or if one is routinely observing multiple positive BIs at multiple locations over many days of testing, then we must consider that there is a true process deficiency in need of attention. But on the contrary, if one is using the triplicate BI approach and is regularly seeing zero positive BIs at all test locations and an unexpected positive does appear, the above analysis should allow the cycle to be passed, regardless of the true cause of the growth-positive result.

Adopting the replicate BI approach as your standard, everyday methodology is somewhat analogous to carrying car insurance. No one ever intends to be involved in an auto accident, nor do we develop processes where we expect to routinely observe growth-positive results. Using triplicate BIs every day (as opposed to only in reaction/investigation) is preparation for the inevitable. If a growth-positive BI does show up, we are already prepared with the statistical analysis to evaluate the situation and no expensive retesting or revalidation will be needed to address this one, unexpected, potentially rogue, growth-positive biological indicator.

US historical figure Benjamin Franklin is credited with coining the phrase, ''an ounce of prevention is worth a pound of cure." In this scenario, every day use of triplicate BIs in your VHP cycle is that "ounce of prevention".

About the author:

Garrett Krushefski is the Manager of International Sales for Mesa Labs. In his twelve years at the Bozeman, Montana location, Mr. Krushefski began his work in the biological indicator production laboratory, later became the Supervisor and eventually the Laboratory Manager. Most recently he provided technical assistance and guidance to customers with respect to sterilization and the proper application of biological indicators as the Scientific & Technical Services Manager.
Mr. Krushefski is a committee member and active participant with the Association for the Advancement of Medical Instrumentation (AAMI) in both the Biological Indicator and Resistometer working groups.
Mr. Krushefski holds a B.A. in Biology from The University of Texas at Austin.

[1.] Drinkwater J, Chewins J, Steele G. Biological indicators don't lie, but in sporicidal gassing disinfection cycles do they sometimes confuse the truth? European Journal of Parenteral & Pharmaceutical Sciences 2009; 14(1): 5-11.
[2.] Templeton P, Hillebrand J. Case Study: Isolator Sanitisation Cycle Development, Validation and Revalidation.
[3.] Technical Report No. 51 Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use, Parenteral Drug Association: Bethesda, MD, 2010.
[4.] Halvorson H, Ziegler N. Journal of Bacteriology 1933; 25, 101-121