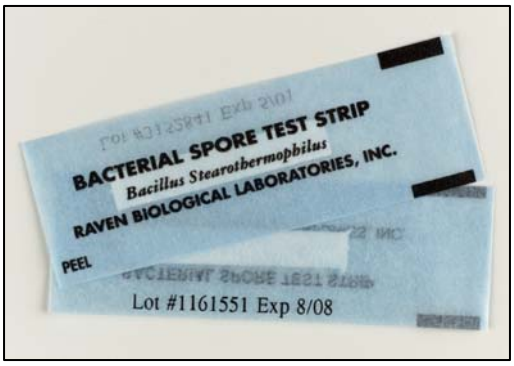
Biological Indicators
D-value and Population Verification/Assessment

By Russ Nyberg

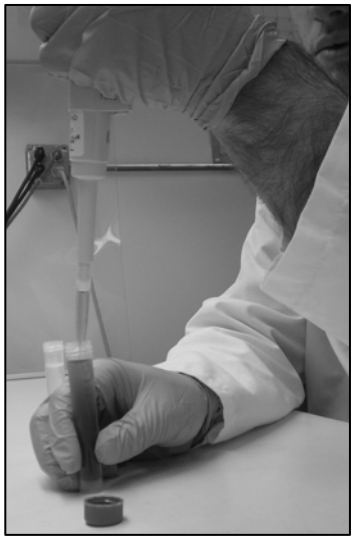
A large number of end-users of Biological Indicators (BIs) are sending the BIs out to 3rd party laboratories for verification of the D-value and/or population of the BI prior to acceptance for use. Having worked within the biological indicator manufacturing industry for nearly 16 years and through hundreds of personal conversations with end users of BIs in regards to BI verification/assessments, it becomes increasingly clearer to me that some very real confusion exists in relation to 3rd party testing and assessment/verification testing results and what can and can not be done with this data.



USP 31, '*User's Responsibility*'[1] states: "*The user should establish in-house acceptance standards for biological indicator lots and consider rejection in the event the biological indicator lot does not meet the established in-house performance standards. A Certificate of Performance should be obtained for each lot of indicators, and the user should routinely perform audits of the manufacturer's facilities and procedures.*"

"*Upon initial receipt of the biological indicator from a commercial supplier, the user should verify the purity and morphology of the purchased biological indicator organisms. Verification of at least the proper genus is desirable. Also, a microbial count to determine the mean count per biological indicator unit should be conducted. The manufacturer's comments relative to D-value range, storage conditions, expiration dating, and stability of the biological indicator should be observed and noted. The user may consider conducting a D-value assessment before acceptance of the lot.*"

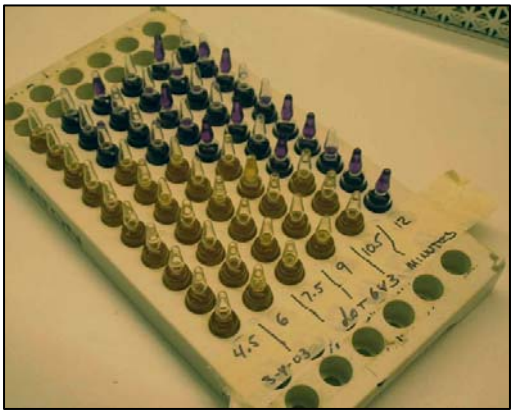
In review of the above USP excerpt, many facilities have instituted in-house acceptance criteria for in-coming BIs prior to allowing them to be used. (*"a microbial count…should be done.")* A large number of end users are doing this. They are performing the population assay's themselves or are sending them out for 3rd party population verification. When D-value is concerned, most facilities do not have the proper equipment to do a D-value assessment so they are sending these BIs off to a 3rd party for testing. USP states that: "*The user may consider conducting a D-value assessment before acceptance of the lot.*" One should note that 'may consider' is not a *must* or *should*. The statement is fairly clear, *may consider*. If one chooses to consider a D-value assessment or the facility protocol requires a D-value assessment prior to use, then a 3rd party testing lab is usually needed. When the test BIs are sent off to the 3rd party testing lab, the end-user should be requesting a D-value assessment using the same D-value testing methods used initially by the BI manufacturer to determine and certify the D-value for that particular Lot of BIs. The methods used by the



[1] USP 31, *General Information*, <1035> Biological Indicators for Sterilization, pg. 401

BI manufacturer should be stated on the certificate of analysis (C of A) provided with the BI Lot. It is important to remember that in a D-value assessment test, you are getting from the 3rd party lab just that, *an assessment.* An *assessment* is not the same as a *determination.*

If the assessment is within the allowable + or – 20% of the certified D-value stated on the manufacturers certificate of analysis, the assessment passes the test and the D-value '*as certified'* on the C of A can be accepted and is to be used whenever that Lot of BIs is used. The certified D-value has been assessed, substantiated, supported or confirmed. Regardless of the terminology used, it is still an assessment. However, it is not to replace the manufacturers certified D-value. Unfortunately in far too many cases this is what is happening. The end user now uses the 3rd party testing labs result for D-value (or population) as the "new, established D-value". This just can not be done. The D-value testing assessment can not replace or be used to re-label the certified D-value determined by the manufacturer. To initially determine and certify the manufacturers stated D-value, methods allowed by ISO and USP were used. As in ISO 11138 series, to obtain a label claim or certify a determined D-value, one must use two of the three methods outlined in ISO and USP[2]: Most Probable Number method by direct enumeration, a Fraction Negative Method (such as Spearman/Karber) or Survive/Kill. Whichever two methods are chosen, two of the three must be used for D-value determination. A 3rd party assessment is not a determination and may not in any way be used to re-label a BIs resistance characteristic certified by the manufacturer.



A similar situation exists with 3rd party population verification. For the BI to pass the population verification, the population result needs to be within +300% and -50% of the labeled population. If the population being verified falls within this range, the requirements of the test are met and the population has been verified. **However, the verified population is not the new certified population to be used in further studies or validations done with this Lot of BIs.** In most situations, BI manufacturers are much more familiar with, have validated and have extended experience in performing population assays on their particular BIs. Having a 3rd party labs assay result replace a manufacturers certified population, even if only slightly different than the C of A listed population makes little sense and is simply not to be done. The bottom line is that 3rd Party verifications are only that, verifications, and are not in any way intended to replace a manufacturer's certified BI characteristics such as D-value or population. Some testing labs are actually performing re-certifications and some end-users are using the 3rd party verification to *replace* the C of A labeled values. If verification criteria are met, the certified characteristics on the manufacturers C of A are to be used. I certainly would not want to be the one who re-labeled a medical device for use in my facility if I were the end-user of a purchased BI. The FDA may even consider this adulteration of a medical device.

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[2] USP, Microbiological Tests, Chapter <55>, Biological Indicators-Resistance Performance Tests. ISO 11138-1 Sterilization of Health Care Products, Biological Indicators Part 1.