MesaLabs

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Spore News

# Verification Testing with Different Media is a Waste of Time

By Garrett Krushefski

I like brownies. One of my favorite things to do is bake brownies with my six-year-old daughter, Kaitlyn. She loves adding the ingredients together and mixing the batter. The smell of the kitchen as they are baking is wonderful. And of course, nothing beats enjoying a freshly baked batch of brownies with a cold glass of milk while the brownies are still warm.

You might think that I'm an accomplished dessert chef, but you'd be wrong. I have to use the boxed mix like most other folks because my talents do not include making anything from scratch. It is fairly easy; all you need to do is **follow the instructions:**

¼-cup of water
½-cup vegetable oil
2 eggs for "fudge-like" brownies, 3 eggs if you prefer "cake-like" brownies
powdered brownie mix

Mix all ingredients (I prefer the fudge-like brownies), spread in a pan, bake as instructed, and voilà, warm decadence is only 28-minutes away.

I never substitute peanut oil for the vegetable oil. I never use milk instead of water. I never bake for longer than recommended. Why? Because I know that **following the manufacturers' instructions** is going to give me (the untalented baker) the best chances to produce a tasty batch of brownies. And trust me, you do not want to have to explain to an anxious 6-year-old that the brownies are not edible because Daddy decided to try something different than that which the manufacturer recommended.

*What's the point?* Glad you asked. The point is:

The same is true for verification testing on biological indicators. Many users have selected to perform population verification testing as part of their incoming inspection requirements. To provide the best chance for successful verification of population label claim, Mesa Labs offers the Population Assay Glassware Kit (catalog number PAK-G), the Growth Media Kit (catalog number PAK-M), and the Population Assay Procedures (available on our website) with detailed step-by-step **instructions** to help the user repeat our test method as closely as possible. The glassware kit contains glass-bead maceration tubes, dilution blanks and pipets. The growth media kit includes a bottle of recovery agar from the same brand/batch of media that was used to determine the value presented on the Mesa Certificate of Analysis. There is significant literature available that dates back at least 40 years which documents the impact recovery media has on both spore count and resistance performance.[1,2,3,4] Refer to Spore News Volume 4, Number 1 for additional discussion.

To further illustrate the need to control all aspects of the testing procedure, the standards have the following recommendations:

**From ISO 11138-1:2017**

With regard to spore population testing, Annex A 3.4 reads, "The biological indicator manufacturer shall identify or make available a suitable medium for recovery of test organisms and/or complete data and instructions for the preparation of such a medium." Regarding D-value testing, Annex D 3.1.1.5 states, "If the growth medium is included by the manufacturer as an integral part of the biological indicator; the manufacturer's culturing instructions shall be followed."

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[1] Pflug, I.J., Smith, G.M., and Christensen, R. 1981. Effect of soybean casein digest agar lot on number of *Bacillus stearothermophilus* spores recovered. *Appl. Environ. Microbiol.* 42 (2): 226-230.

[2] Shintani, H., Sasaki, K., Kajiwara, Y., Takahashi, M., Kukobo, M. 2000. Validation of D-value by Different SCD Culture Medium Manufacturer and/or Different SCD Culture Medium Constituent. *PDA Journal of Pharm. Sci. & Tech.,* Vol. 54, No. 1, pp. 6-11.

[3] Boris, C., and Graham, G.S., 1985. The effect of recovery medium and test methodology on biological indicators. *Med. Device Diagn. Ind.* 7 (2): 43-48.

[4] Davis, S.B., Carls, R.A., and Gillis, J.R. 1979. Recovery of sublethal sterilization damaged Bacillus spores in various culture media. *Developments in Industrial Microbiology*, Vol 20. Underkofler, Leland A., and Wulf, Margaret L., pp. 427-438. Arlington Va: Society for Industrial Microbiology.

**From ISO 11138-7:2019**

"When tested, the population should be between 50% and 300% of the nominal population... The biological indicator manufacturer should be consulted to ensure that the same techniques and procedures are used because variations in testing procedures can affect the population determination results...Different laboratory practices and even variations in the performance of the individual personnel can lead to different results…The user should follow the manufacturer's recommended procedures for recovery to ensure comparable results."

The ISO 11138 series limits deviations from the labeled nominal number.[5] The user should note that if the nominal population of an indicator from a given batch or lot of indicators is being tested by the user, the deviations can exceed the limits given in the relevant part(s) of ISO 11138. This could be caused, for example, by different culture media used or different enumeration and counting techniques.

**From PDA Technical Report No. 1 (Revised 2007)**

3.2.1 "It is important to use the same enumeration methodology that the vendor used in order to minimize variables that could lead to differences in spore counts."

These were the driving forces that resulted in development of the Population Assay Glassware Kit and the Growth Media Kit. Scientists know that one should always eliminate as many potential sources of variation as possible when attempting to verify or reproduce data. Use of these kits is the ONLY way to ensure this as the kits eliminate and minimize as many variables as possible. As suggested in the above ISO & PDA citations, failure to eliminate these sources of variation increases one's chance to obtain non-compliant results.

The population assay test is one of the few tests in which a microbiology laboratory technician is attempting to confirm a quantitative value. All analysts are confident in their technique and, therefore, believe their data accurate. When this test is performed only a few times each year there is little doubt that performance in execution is not nearly as good as the test manipulations that are performed on a daily basis.

Don't be creative and get caught wasting your time and efforts by deviating from the specified test method. Doing so often results in costly retests and delays… which leaves less time for milk and brownies. Time to go…my 28-minute timer is about to ring!

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[5] ISO 11138-1:2017, 6.3.2 Population verification shall be achieved when results fall within 50% to 300% of the manufacturer's stated nominal population. Confirmation test results of the population determined by end users or manufacturers during stated shelf life may meet the 50% to 300% range but could fall below the minimum population specification as defined in this document. In these cases, the original population is considered to be verified if the confirmation test results are within the 50% to 300% range.