MesaLabs

Vol. 10 No. 2
Spore News

Total Viable Spore Counts

By Robert Bradley

Total viable spore count testing is a common in-coming inspection test for biological indicator (BI) users. This test serves as one of the simpler tools that an end user can use to ensure the quality of the product they are receiving. As an end user prepares to perform this test, they might ask the question “what procedure should I use?”.

The answer might not be as straight forward as you would expect. There isn’t a ‘one size fits all’ answer. Both the United States Pharmacopeia (USP) and the International Organization for Sterilization (ISO) list procedures that vary from one another for determining viable spore counts and most BI manufacturers have developed procedures of their own that are specific to their product variations. So, what is the right answer? Let’s look at the standards and manufacturer specific procedures first and see what we turn up.

USP <55> provides the most detailed procedure of the two standards. USP requires a minimum of four test samples. USP outlines three different procedures: paper/fiber indicators, indicators on other substrates and spore suspensions. Of these three procedures, only spore suspensions varies at the first step, eluting or removing the test organism from the test sample. For this article we will focus on the paper/fiber indicators and indicators on other substrates.

1. Elute the test organism from the test samples. Disperse the indicator into component fibers by placing the four test samples into a sterile vessel containing 100ml of sterilized purified water, chilled to 2 - 8°C and mechanically disrupt to achieve a homogeneous suspension.

2. Transfer a 10ml aliquot to a sterile tube.

3. Heat shock the tube at the appropriate temperature, starting the timing when the temperature reaches the heat shock temperature.
    - Thermophiles: 95-100°C for 15 minutes
    - Mesophiles: 80-85°C for 10 minutes

4. Remove from heat shock and rapidly cool in an ice-water bath at 0-4°C.

5. Transfer two 1ml aliquots to suitable tubes and make appropriate serial dilutions in sterile purified water. The selected dilutions should be those that will yield 30-300 colonies.

6. Plate 1ml of each selected dilution into each of two Petri dishes.

7. Within 20 minutes, add 20ml of agar that has been melted and cooled to approximately 45°C. Allow to solidify.

8. Invert the plates and incubate at the appropriate temperature for 48 hours.

ISO 11138-1 is more vague than USP, it just provides a basic outline of a procedure. ISO also requires a minimum of four test samples. It states that the test samples should be placed in an appropriate volume of suspending medium and that the test organism should be eluted from the test samples by a validated procedure.

Once the test organism is eluted from the samples then proceed with serial dilutions and plating in/on tryptic soy agar plates. Colony counts of 30-300 are considered the most accurate. ISO is relying on the BI manufacturers to provide the end user with the appropriate procedure for their different types of BIs.

Even though there are procedures outlined in USP you will find that a lot of BI manufacturers have validated different variations of the base procedure listed in USP. Typically, this is done to increase efficiency as well as accuracy. Mesa Labs has validated variations to the USP procedure.

Mesa ‘s procedure for paper/fiber carriers very closely follows the USP procedure with two exceptions:

- Maceration of the paper/fiber carrier in 10 mL of sterile purified water, in a 19.5 x 145 mm flat bottom tube, containing four 6 mm glass beads.
- Beginning the timing of the heat shock immediately when the tubes are placed into the water bath.

The changes listed above work very well for the specific manufacturer that employs them but that doesn't mean they should be used universally for all BIs from all manufacturers. The manufacturers produce their organisms and biological indicators using different methods. Those methods could cause the organisms they use for their BIs to react differently to a procedure that has been validated by another manufacturer. As an example, the glass bead maceration that Mesa utilizes works very well for spore strips manufactured there but if you were to utilize that method for other manufacturer's spore strips , you might find that it may not work as well if the manufacturer utilizes a thicker paper which doesn't macerate as well using glass beads.

So, what is the answer? What procedure should you follow? Ideally you should use the same procedure as the manufacturer. You are trying to replicate the manufacturer's results, and the best way to do that is to follow the same procedure. Most manufacturers will gladly provide you with their standard operating procedure for performing total viable spore counts.
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About the Author

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Mr. Bradley holds a B.S. in Biology from Midland Lutheran College and a M.S. in Biology from the University of Nebraska at Omaha. He is a member of the Association for the Advancement of Medical Instrumentation (AAMI), the Parental Drug As- sociation (PDA), and the American Society for Microbiology (ASM).