Method for Population Verification of Spore Ampules

This Technical Bulletin outlines the procedure recommended for use in verifying the certified population of True Indicating Spore Ampules. This bulletin applies to 1.1 mL Spore Ampules of *Geobacillus stearothermophilus* with populations of 10^4/ampule through 10^6/ampule.

1. Obtain sterile 20 mm x 150 mm test tubes (or equivalent of sufficient size to hold a minimum of 25 mL) in the appropriate quantity to accommodate the required dilution series.

   Use of a minimum of 4 Ampules is outlined in ISO 11138-1 Sterilization of health care products – Biological indicators – Part 1: General requirements.

2. Fill test tubes with 9 or 9.9 mL of Sterile Deionized Water (SDI) or Water For Injection (WFI) to meet requirements of the determined dilution series.

3. Vortex or manually shake the test Spore Ampule to ensure the spores are evenly dispersed throughout the ampule. Holding the Ampule upright by the base, apply pressure to snap the top of the ampule to open it. Transfer 0.1 mL of suspension to 9.9 mL of diluent to achieve a 10^{-2} dilution.

Heat Shock

4. Prepare a "blank" test tube containing 10 mL of the diluent only (WFI or SDI). Place a thermometer in the "blank" test tube.

5. Place the test tube(s) containing the initial dilution (10^{-2}) and the "blank" into a water bath.

6. Start timing the length of the heat shock period when the thermometer reaches 95ºC. Continue the heat shock period for 15 minutes in the range of 95ºC to 100ºC.

Organism	Heat Shock Temperature	Length of Heat Shock Period
Geobacillus stearothermophilus	95ºC to 100ºC	15 minutes

7. At the end of the heat shock period, transfer the tubes from the water bath to an ice bath and allow to cool at 0º - 4ºC.

Dilution and Plating

8. Perform dilutions (1:10 = 1 mL into 9 mL diluent or 1:100 = 0.1 mL into 9.9 mL diluent) until the dilution corresponding to the theoretical population of 30 to 300 spores/mL is achieved.

9. Obtain 100 mm x 15 mm petri dishes. Label each petri dish with Ampule number and dilution. A minimum total of 2 transfers to petri dishes or two plates per Ampule is recommended.

10. Transfer a 0.1 mL or 1 mL aliquot based on the dilution series created from the final dilution tube of each Ampule into separate dishes as per labeled above.

11. Within 20 minutes, add approximately 20 mL of molten Soybean Casein Digest Agar (SCDA)/Tryptic Soy Agar (TSA) to each dish and mix by gently swirling. The temperature of the media is a critical factor as media which has not been properly tempered will damage and/or kill the spores thus reducing the recovery. Ensure media is approximately 45ºC when poured into the petri dishes.

12. Allow the agar to solidify.

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Incubate

13. Invert the petri dishes and incubate for a minimum of 48 hours at 55ºC to 65ºC.

Enumerate

14. After incubation, enumerate the colonies on each plate and calculate the overall mean count based on the average of the results for each Ampule. See example below for guidance:

    Based on the dilution factor of 10^{-4} plated at 1 mL, calculate the total viable spore count.

Ampule No.	Plate 1	Plate 2	Plate 3	Average
1	152	140	165	152
2	180	141	191	171
3	172	163	153	163
4	144	182	196	174

    Overall Mean: 165

15. Multiply the Overall Mean by 1.1 based on the volume of the Spore Ampule (which is 1.1 mL) to obtain the Total Viable Spore Count per Ampule:

    165 x 1.1 = 181.5 or 1.8 x 10^6/Ampule

Acceptance Criteria

16. Per ISO 11138-1, the population should be within 50% to 300% of the certified population (manufacturer's label claim) to be considered acceptable. A Lot of Spore Ampules with a certified population of 1.8 x 10^6/Ampule, would be acceptable if the verified average population was in the range of 9.0 x 10^5 to 5.4 x 10^6/Ampule.