Method for Population Verification of Dry Heat Ampules

This Technical Bulletin outlines the procedure recommended for use in verifying the certified population of True Indicating Dry Heat Spore Ampules of *Bacillus atrophaeus* Product Code AAD-06.

1. Obtain one sterile 20 mm x 150 mm screw cap test tube (or equivalent) containing approximately 1" or 2.54 cm of sterile 3 mm glass beads for each Spore Ampule to be evaluated.

   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. Holding the Ampule upright by the base, apply pressure to snap the top of the ampule to open it. Transfer contents of Ampule to tube. Add 10 mL of Purified Water (PW), Water For Injection (WFI) or Sterile Deionized Water (SDI) diluent to achieve a 10^{-1} dilution.

Maceration or Dislodging of Spores

3. Vortex the tube for a minimum of 3 minutes.

Heat Shock

4. Transfer 1 mL of the fluid to 9 mL of PW, WFI or SDI (this is the 10^{-2} dilution).
5. Prepare a "blank" test tube containing 10 mL of the diluent only (PW, WFI or SDI). Place a thermometer in the "blank" test tube.
6. Place the test tube(s) and the "blank" into a water bath.
7. Start timing the length of the heat shock period when the thermometer reaches the organism's minimum heat shock temperature as outlined in the table below. Continue the heat shock period for the time specified.

Organism	Heat Shock Temperature	Length of Heat Shock Period
Bacillus atrophaeus	80ºC to 85ºC	10 minutes

8. 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

9. Based on the target population level of the carrier (10^6), perform 1:10 dilutions (1 mL into 9 mL diluent) or 1:100 dilutions (0.1 mL into 9.9 mL) until the 10^{-4} dilution corresponding to the theoretical population of 30 to 300 spores per mL is reached.

10. Obtain 100 mm x 15 mm petri dishes. Label each petri dish with Carrier number, the dilution factor and the plate number. A minimum total of 2 transfers to petri dishes or two plates per Carrier is recommended.

11. Transfer a 1 mL aliquot from the final dilution tube of each carrier into separate dishes as per labeled above.

12. 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.

13. Allow the agar to solidify.

Incubate and Enumerate

14. Invert the petri dishes and incubate for a minimum of 48 hours at 30ºC to 40ºC.
15. After incubation, enumerate the colonies on each plate and calculate the overall mean count based on the average of the results for each carrier.
16. Calculate the total viable spore count. See example below for guidance:

    Example: 10^{-4} Dilution

Ampule/Carrier No.	Plate 1	Plate 2	Plate 3	Average
1	152	140	165	152
2	180	141	191	171
3	172	193	153	173
4	144	182	196	174

    Overall Mean: 167.5 = 168
    Total Viable Spore Count: 1.7 x 10^6/Ampule

Acceptance Criteria

17. 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 Dry Heat Ampules with a certified population of 1.7 x 10^6/carrier, would be acceptable if the verified average population was in the range of 8.5 x 10^5 to 5.1 x 10^6/carrier.