Technical Bulletin

Population Verification for Biological Indicator Mini Strips (2 mm x 10 mm), Discs (1.0 cm, 3 mm and 6 mm) and Threads

This technical bulletin outlines the laboratory procedures used by Excelsior Scientific to verify the labelled spore population of biological indicators including Mini-Strips, Discs, Wires, Threads & Steel Coupon product configurations.

Suitable for product codes: STN-062E, STN-062BE, STS-062E, STS-062BE, DN-06E, DN18-06E, DS-06E, DS18-06E, SDN-06E, SDS-06D, TTS-06E and MISC codes for Threads or glass fiber product configurations. .

Spore Recovery

1. Remove the inoculated carrier (Strips, disc, thread, wire, coupon) from the packaging materials if applicable.
2. Transfer the inoculated carrier into a sterile 20 mm x 150 mm screw cap test tube (or equivalent). Add 10 ml of purified water (PW), water for injection (WFI) or sterile distilled water (SDI) and enough sterile 5 mm glass beads to fill the test tube to the level of the water within the tube. For filter paper carriers (mini-strips and discs), macerate the carrier through agitation by using either a mechanical vortex or by manually shaking the test tube until a homogeneous pulp is obtained.

   For carriers other than paper, including glass fibre discs, threads & wires, vortex or sonicate the tube for a minimum of 20 minutes to completely dislodge the spores from the carrier.
   NOTE: The preferred method for dislodging spores is through sonication. Where possible sonicate at a minimum of 67 khz.

Heat Shocking

3. After maceration or dislodging of the spores, do not transfer or dilute maceration fluid from original tube prior to performing the heat shock process.
4. Prepare a “blank” test tube containing 10 ml of the diluent only (PW, WFI or SDI). Place a thermometer in the “blank” test tube.
5. Place the test tube(s) including the maceration fluid and “blank” into a water bath.
6. Start timing the length of the heat shock period when the thermometer reaches 80°C for heat shocking of Baciluss atrophaeus or when the thermometer reaches 95°C for Geobacillus stearothermophilus as follows:

Organism	Heat Shock Temperature	Length of Heat Shock Period
Bacillus atrophaeus	80°C to 85°C	10 minutes
Geobacillus stearothermophilus	95°C to 100°C	15 minutes

7. After the heat shocking period is complete, cool the tube rapidly in an ice water bath (0° to 4°C.

Dilution and Plating

8. Perform a 1: 10 dilution series until a dilution corresponding to a theoretical population of 30 to 300 spores per ml is reached. The maceration fluid in the test tube is dilution tube 10^{-1} as each 1 ml aliquot is 1/10th the original BI population. For products inoculated with 10^6 spores, perform the dilution series to 10^{-4} to achieve between 30 and 300 spores per mL.
9. Transfer 1 mL aliquots from the final dilution tube into separate 100 mm x 15 mm Petri dishes.
10. Within 20 minutes add 20 mL of the molten Soybean-Casein Digest Agar (SCDA) or equivalent (melted in boiling water and cooled to a temperature of 45°C or incubated (stored) at 45°C) to each plate and mix by gently swirling. The temperature of the media is a critical factor as media that has not been properly tempered (too hot) will damage and /or kill the spores thus reducing the recovery.

Incubate and Enumerate

11. After the agar has solidified, invert the plates and incubate for a minimum of 16 hours at the appropriate growth temperature (30-35°C for *Bacillus atrophaeus* and 55-60°C for *Geobacillus stearothermophilus*).
12. Enumerate colonies and calculate the overall mean count based on an average of the results from each plates.
13. Based on the dilution factor, calculate the total viable spore count as outlined in the following example:

Example: 10^{-4} Dilution

	Plate #1	Plate #2	Plate #3	Plate #4
Plate 1	152	140	189	165
Plate 2	180	141	190	192

Overall Mean Count: 168.6 = 169
Total Viable Spore Count: 1.7 x 10^6 / carrier

For additional product information:
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Acceptance Criteria

14. Per ANSI/AAMI/ISO 11138-1:2006, the population shall be within 50% to 300% of the certified population (manufacturer's label claim) to be considered acceptable.

    Based on the above example the acceptable population range for the subsequent verification testing would be 0.85 x 10^6 to 5.1 x 10^6 / carrier per ANSI/AAMI/ISO 11138-1:2006

NOTES:

1. Use sterile materials and aseptic technique throughout.
2. Test diluent and culture media for growth support or employ suitable positive controls (such as previously qualified BI's).

For additional product information:
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