This Technical Bulletin outlines the procedure recommended for use in verifying the certified population of True Indicating Mini-Spore Strips (2 mm x 10 mm). This bulletin applies to Mini-Spore Strips of *Bacillus atrophaeus* and *Geobacillus stearothermophilus*.

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 Mini-Spore Strip to be evaluated.

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

2. Remove the inoculated carrier from the packaging material, if applicable, and add to the sterile test tube with glass beads. Add 10 mL of Purified Water (PW), Water For Injection (WFI) or Sterile Deionized Water (SDI).

Maceration

3. Macerate the carrier through agitation using either a vortex, manually shaking the test tube or a combination of the two until a homogeneous pulp (only fibers present in tube, no chunks of the strip remain) is obtained. This process will take approximately 5-10 minutes; longer if the tube is only vortexed.

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
Geobacillus stearothermophilus	95ºC to 100ºC	15 minutes

Dilution and Plating

8. Perform 1:10 dilutions (1 mL into 9 mL diluent) or 1:100 dilutions)1 mL into 9.9 mL diluent) until the 10^{-4} dilution is reached.

9. Obtain 100 mm x 15 mm petri dishes. Label each petri dish with Inoculated Carrier number, the 10^{-4} dilution factor and the plate number. A total of 3 transfers to petri dishes or three plates per carrier is recommended.

10. Transfer a 1 mL aliquot from the final dilution tube (10^{-4}) of each carrier 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.

True Indicating LLC.
946 Kane St., Suite A, Toledo OH 43612
www.trueindicating.com

Contact us at: info@trueindicating.com

Incubate and Enumerate

13. Invert the petri dishes and incubate for a minimum of 48 hours at the appropriate growth temperature for the organism, as outlined below:

Organism	Incubation Temperature
Bacillus atrophaeus	30°C to 40°C
Geobacillus stearothermophilus	55°C to 65°C

14. After incubation, enumerate the colonies on each plate and calculate the overall mean count based on the average of the results for each carrier.

15. Based on the dilution factor of 10^{-4}, calculate the total viable spore count. See example below for guidance:

    Example: 10^{-4} Dilution

Mini-Spore Strip / 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: 1.7×10^6/strip

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 Mini-Spore Strips with a certified population of 1.7×10^6/strip, would be acceptable if the verified average population was in the range of 8.5×10^5 to 5.1×10^6/strip.