CROSSTEX®
A CANTEL MEDICAL COMPANY

TECHNICAL BULLETIN

Population Verification of Spore Suspensions

This technical bulletin outlines the laboratory procedures used by Crosstex to verify the labeled spore population of Spore Suspensions.

Use sterile materials and aseptic technique throughout the verification procedure.

1. Vortex the vial for 90 seconds.
2. Use a sterile pipette or syringe to accurately measure the volume of suspension to be diluted. Serially dilute the sample using sterile water (WFI) until a theoretical population of 30 – 300 spores per mL is reached, vortexing or vigorously shaking the tube containing the diluted sample prior to each transfer. Reserve a minimum of 1 mL of WFI for sterility verification.

   As an example for preparation of dilutions, a product with a population level of 10^6 per 0.1 mL would require a 10^{-5} dilution to achieve between 30 and 300 spores per mL.
3. Heat shock the final dilution tube according to the following table. Prepare a control tube for the heat shock procedure by adding an appropriate volume of water to a test tube, equivalent to the volume contained in the dilution tube. Place a calibrated thermometer in the tube. Simultaneously place the control tube and the sample dilution tube into a preheated water bath. Begin timing the heat shock period when the thermometer reaches the minimum temperature.

Organism	Heat Shock Temperature	Heat Shock Duration
Bacillus atrophaeus	80 – 85°C	10 minutes
Bacillus pumilus	65 – 70°C	
Bacillus subtilis (Cell Line 5230)	80 – 85°C	
Bacillus subtilis (Cell Line 6633)		
Geobacillus stearothermophilus	95 – 100°C	15 minutes

4. Remove the tubes from the water bath and immediately cool.
5. Vigorously shake the final dilution tube and transfer 0.5 mL or 1.0 mL to a properly labeled 100 x 15 mm petri dish in duplicate.
6. Overlay each sample with 30 – 35 mL of molten tryptic soy agar which has been equilibrated to 45 – 50ºC.

   Crosstex recommends the use of BD BBL™ Trypticase® Soy Agar (ref: 211043) dehydrated media which has been prepared per the manufacturer's instructions and tested for growth promotion capabilities. Media which has not been properly tempered will damage and/or kill the spores, thus reducing the recovery.
7. Gently swirl each plate to evenly distribute the spores.
8. Prepare negative control plates for the media and reserved diluent. Pour 30 – 35 mL of agar into an empty petri dish as the media control. Overlay the reserved volume of WFI with 30 – 35 mL of agar as the diluent control.

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9. Allow agar to solidify at room temperature. Invert and incubate all plates at the appropriate growth temperature for minimum of 48 hours.

Organism	Incubation Temperature
B. atrophaeus	35 – 39°C
B. pumilus	
B. subtilis (Cell Line 5230)	
B. subtilis (Cell Line 6633)	
G. stearothermophilus	55 – 60°C

10. Enumerate the colony forming units (CFUs). Counts within the range of 30 – 300 CFUs per plate are considered acceptable. If necessary, repeat the procedure with an alternative dilution volume to achieve the appropriate range.

11. Calculate the average CFUs per mL and multiply by the dilution factor to obtain the suspension population.

Example:
Suspension population: 10^6 per 0.1 mL
Dilution: 10^{-5} (Dilution factor = 100,000)
Volume per plate: 0.5 mL

Plate 1 CFUs	Plate 2 CFUs	CFUs/mL	Population per mL	Population per 0.1 mL
72	56	128	1.3×10^7	1.3×10^6

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

Trypticase® is a registered trademark owned by Becton, Dickinson and Company.
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