Assay Instructions

ProSpore 4mL Ampoules

I. List of Components:

Mesa Labs, Bozeman Manufacturing Facility sells components for performing population assays. These include:

PAK-G includes: four 19.5 x 145 mm, sterilized, flat bottom glass tubes with four 6 mm beads and cap; twelve 16 x 125 mm, sterilized, borosilicate dilution blank tubes; two 10 mL pipettes; two 5 mL pipettes; eight 2 mL pipettes; eight 1 mL pipettes

PAK-M includes: one 250 mL Wheaton bottle containing 240 mL of sterile Difco brand growth medium

Items required are: growth medium, sterilized flat-bottom tubes with four 6mm beads, sterilized blank tubes for dilution, pipettes, 160 mL purified sterile water*, a pre-heated (according to Table 1) heat-shock bath and incubator, an instrument used for holding the melted growth medium at 45 - 50 ˚C, a timing device, a vortex machine, an ice bath, and 15 x 100 mm petri plates. An ultrasonic cleaner (45-60 kHz) is required when assaying ProSpore ampoules.

*Throughout this procedure when sterile purified water is referenced this includes; Sterile distilled, DI or RO water. WFI, phosphate buffers or physiological saline solutions are not recommended.

II. Preparing the Growth Medium for use:

NOTE: If you have purchased growth medium from Mesa Labs, the medium was prepared according to Good Manufacturing Practices (GMP), and has been tested for sterility and its growth promotion ability (see Certificate of Performance).

1. The growth medium must be completely melted prior to use. This can be accomplished by using a microwave oven. CAUTION: Melting agar presents a significant risk of explosion if not performed properly. It is important to loosen the screw cap on the bottle prior to placing into the oven. This will prevent pressurization of the bottle. Recommended power setting and operating time will vary depending on the oven type; however, the oven should ONLY be operated at LOW POWER SETTINGS.

2. When completely melted, the agar should be tempered at 45 to 50 °C until ready for use.

3. A control plate should be poured with each assay. The purpose of the control plate is to verify the sterility of the growth medium. The control plate should be prepared upon completion of the assay and it consists of pouring the remaining growth medium into a sterile Petri plate. The control plate should be incubated with the plates from the assay and should result in no growth.

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III. ProSpore Population Assay (method for assaying liquid carriers):

1. Randomly select four ampoules from the lot to be assayed.

2. Vortex each ampoule for one minute in the Upside-down Position, and then for one minute in the Upright Position being careful to wash out any spores that may be adhering to the glass in the upper tip of the ampoule.

   2.1 Safety goggles should be worn as a precaution.

   2.2 Hold the body of the ampoule in one hand and the top of the ampoule in the other hand.

   2.3 Position thumb tips spread away from the scored line of the ampoule. The first knuckle of each thumb should touch, acting as a hinge.

   NOTE: Laceration can occur if thumb tips are touching along the scored line.

3. Aseptically transfer a 1 mL aliquot from each ampoule to a sterile, screw-capped, 19.5 x 145 mm flat bottomed tube containing 9 mL of sterile processed water.

4. In a pre-heated bath, heat-shock this tube according to the test organism (see Table 1) starting the timing immediately upon insertion of sample into the preheated bath.

5. Remove tube and cool rapidly in ice bath.

6. Dilution Series for a 10^5 and 10^6 population:

   Two dilution series will be made from the heat-shocked tube. NOTE: It is extremely important to make each serial transfer immediately after vortexing. Vortex the heat-shocked tube for at least 10 seconds. Using a 1 mL pipette, transfer a 1 mL aliquot to a dilution blank containing 9 mL of sterile purified water. Vortex the dilution tube for at least 10 seconds. Use a 1 mL pipette to transfer 1 mL to a second dilution blank containing 9 mL of sterile purified water. **Repeat this step one more time with a 1 mL pipette for a 10^6 population.** Vortex this tube for at least 10 seconds. From this dilution tube, use the 2 mL pipette to withdraw 2 mL. Pipette 1 mL per plate into two 15 x 100 mm Petri plates (see Figure 1 below). Pour approximately 20 mL of melted growth medium cooled to 45 to 50 ºC into the Petri plates. Swirl to ensure adequate mixing and allow the agar to solidify. Do not use agar that has been melted and held longer than eight hours. From the heat-shocked tube, repeat the above dilution sequence one additional time.

7. Allow to solidify then invert and incubate plates according to test organism (see Table 1).

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8. After 48 hours of incubation, remove the plates from the incubator and count the colony forming units (CFU) on each plate. Preferably plates with counts between 30 and 300 CFU should be used, per ISO and USP.

9. Average the counts and multiply by the inverse of the dilution factor.

10. Multiply this number by the fill volume to give you the population of spores per unit. The fill volume used to calculate the average population for a ProSpore 4 mL ampoule is 3.7 mL

Figure 1

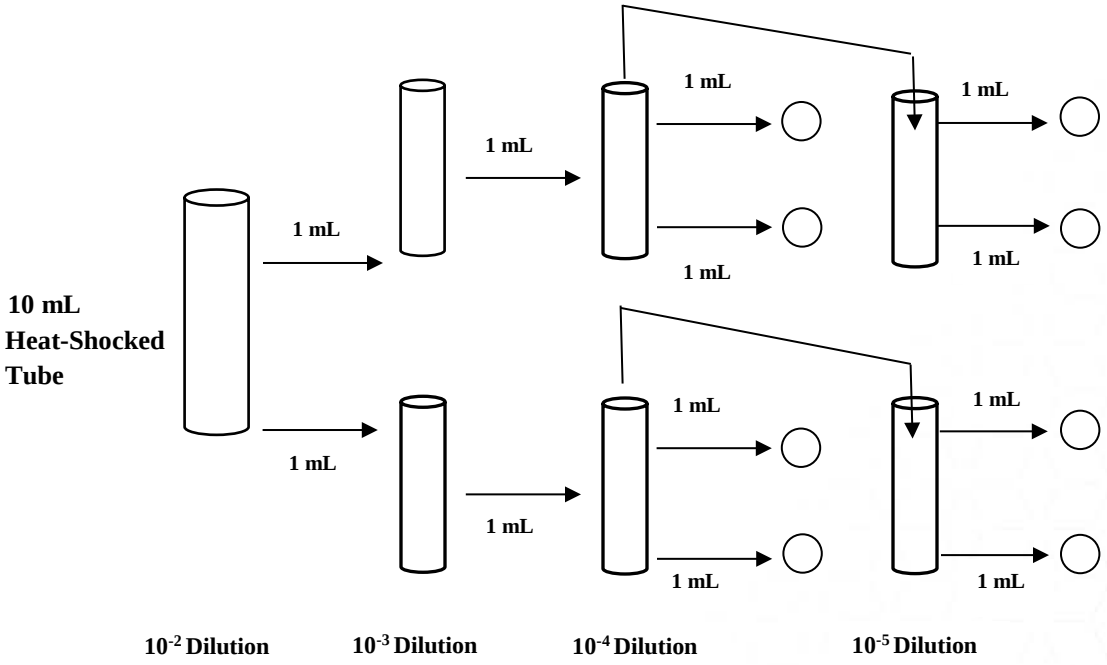

Table 1. Heat Shock and Incubation Temperature for Various Species

Species	Process	Heat shock temperature/time**	Product	Incubation temperature*
Geobacillus stearothermophilus	Steam	95 - 100°C for 15 minutes	ProSpore	55 - 60°C

* Bag plates to avoid dehydration of media at this temperature.
** Start timing immediately upon insertion of sample into preheated bath.

REFERENCE DOCUMENTATION:

LP-301 Population Assay of SterilAmp II, MagnaAmp, and ProSpore Products (Based on)