Population Assay Instructions

Paper, Quartz, & Cotton Thread Carrier Products

I. List of Components:

Mesa Laboratories, Inc., Sterilization and Disinfection Control – Bozeman sells components for performing population assays. These include:

PAK-G includes: four 19.5 x 145 mm, sterilized, flat bottom glass tubes with 4 - 6mm 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 15x100 mm petri plates.

*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. Paper, Quartz, & Cotton Thread Carrier Population Assay method:

NOTE: To avoid inaccurate colony counts, it is important not to allow the pipette tip to become clogged with fibers during the initial transfer from the glass bead tubes.

1. Use one 5-mL pipette to transfer 5-mL of sterile purified water into each 19.5 x 145-mm, flat-bottom tube (containing the four glass beads).

2. Use one 10-mL pipette to transfer 9-mL of sterile purified water into each 16 x 125-mm dilution blank tube.

3. Randomly select four inoculated carriers from the lot to be assayed. See Appendix 1 Removing the Paper Carrier from EZTest.

4. Place one carrier per screw cap 19.5 x 145-mm, flat-bottom tube.

5. Vortex until the carrier is macerated to pulp (cotton thread carrier will not macerate to pulp), Depending on the carrier, generally between four to seven minutes.

6. Use the second 5-mL pipette to add an additional 5-mL of sterile purified water to each macerated carrier. Vortex for 30 seconds.

   NOTE: When adding volumes of sterile water to vortexed units in flat-bottomed tubes, be careful not to contaminate the tip of a pipette by touching it to a receiving tube.

7. In a pre-heated bath, heat-shock each 19.5 x 145-mm flat bottom tube according to the test organism (see Table 1) starting the timing immediately upon insertion of sample into the pre-heated bath.

8. Remove tubes and cool rapidly in ice bath.

9. Dilution series for a 10^5 and 10^6 population:

   A dilution series will be made from each 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 2-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. Pour approximately 20-mL of melted growth medium cooled to 45° to 50ºC 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. Repeat the above dilution sequence for the remaining three heat-shocked tubes.

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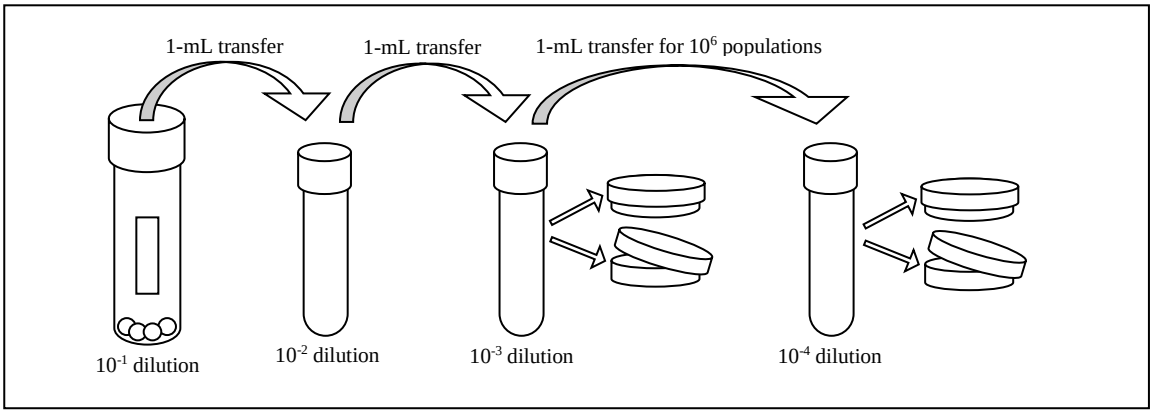

10. Pour control plate.

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

12. 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.

13. Average the counts and then multiply by the inverse of the dilution factor to calculate the population per original unit.

14. Document all information.

Table 1. Heat-shock and Incubation Temperatures for Mesa Laboratories, Inc., Sterilization and Disinfection Control – Bozeman Biological Indicator Test Organisms

Test Organism	Heat shock**	Incubation
G. stearothermophilus	95 - 100°C for 15 minutes	55 - 60°C for 48 hours*
B. atrophaeus	80 - 85°C for 10 minutes	30 - 35°C for 48 hours
B. subtilis '5230'	80 - 85°C for 10 minutes	30 - 35°C for 48 hours
B. subtilis subsp. spizizenii '6633'	80 - 85°C for 10 minutes	30 - 35°C for 48 hours
B. smithii	95 - 100°C for 15 minutes	48 - 52°C for 48 hours*
B. pumilus	65 - 70°C for 15 minutes	30 - 35°C for 48 hours

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

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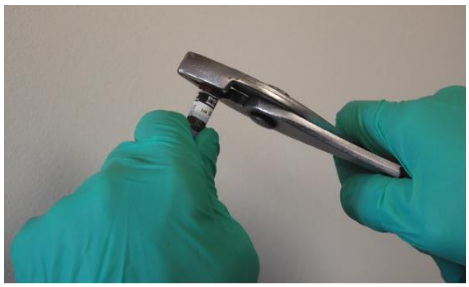
Appendix 1: Removing the Paper Carrier from EZTest

1. Firmly grasp the base of the EZTest in one hand. While a firm grip is necessary, excessive pressure could cause the media ampoule to break. If this occurs, the EZTest will need to be killed and discarded; one should not attempt to assay the paper carrier if it has become saturated with media from the glass ampoule.

2. Grasp the cap of the unit in the other hand and use a repetitive back-and-forth twisting motion as you attempt to pull the cap off the base of the EZTest. Using pliers will greatly facilitate this process; one may find it impossible to remove the cap without aid from a tool (see photo).



3. Once the cap has been removed the filter material may have remained in the cap or it may still be on the EZTest unit. If the latter, remove the filter material.

4. The glass media ampoule should easily fall out of the EZTest when turned upside down. If not, gently tap the open end of the EZTest on the bench top to aid removal of the glass media ampoule.

5. Use sterile forceps to extract the inoculated paper carrier from the EZTest.

REFERENCE DOCUMENT:

LP-305 Population Assay of Biological Indicator Products (Based on)