Population Assay Instructions
Apex Products

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, sterile, flat bottom glass tubes with 4 - 6mm beads and cap; twelve 16 x 125 mm, sterile, 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 glass Wheaton bottle of sterile Difco brand growth medium

Items required are growth medium, sterile flat-bottom tube with four 6mm beads, sterile blank tube 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.

NOTE: When adding volumes of sterile fluid (water, Tween 80 (0.1%) or Fluid D) to vortexed units in flat-bottomed tubes, be careful not to contaminate the tip of a pipette by touching it to a receiving tube.

*Throughout this procedure when sterile purified water is referenced this includes; Sterile distilled, DI or RO water. WFI is 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.

Population Assay Instructions
Apex Products

III. Apex BI Population Assay:

NOTE: There are two acceptable methods for performing population assays for the Apex biological indicator (BI) product. The first one is the method used in the internal testing lab. The second method has been validated to confirm that it provides comparable results.

OPTION 1:

1. Aseptically remove glass beads from the 19.5 x 145mm tubes. Place four ribbons or discs (randomly selected inoculated carriers) individually into the sterile glass tubes (19.5 x 145mm). Add 10mLs of sterile distilled water.

   NOTE: Ribbons should be curved on the inoculated end so that the inoculated part faces down toward the sonic energy.

2. Do not place more than one BI in each tube.

3. Sonicate not less than 15 minutes.

   3.1 De-gas the sonic bath by turning on for 5 minutes before placing the tubes in the wire rack.

   3.1 Rack should be all metal, preferably stainless steel.

   3.2 Tubes should be equidistant from each other.

   3.3 Ensure the rack in the sonic bath is suspended above the bottom of the bath, so that sonic energy reaches the test tubes uniformly.

   3.4 Move tubes to different locations within the rack halfway through the sonication process.

4. Complete the required dilution scheme for each sample to obtain an estimated 30-300 CFU/plate. The dilution scheme represented below is for a carrier inoculated with ≥1e6 spores.

5. Vortex the sonicated tube and BI immediately before beginning the dilution process for not less than 5 seconds.

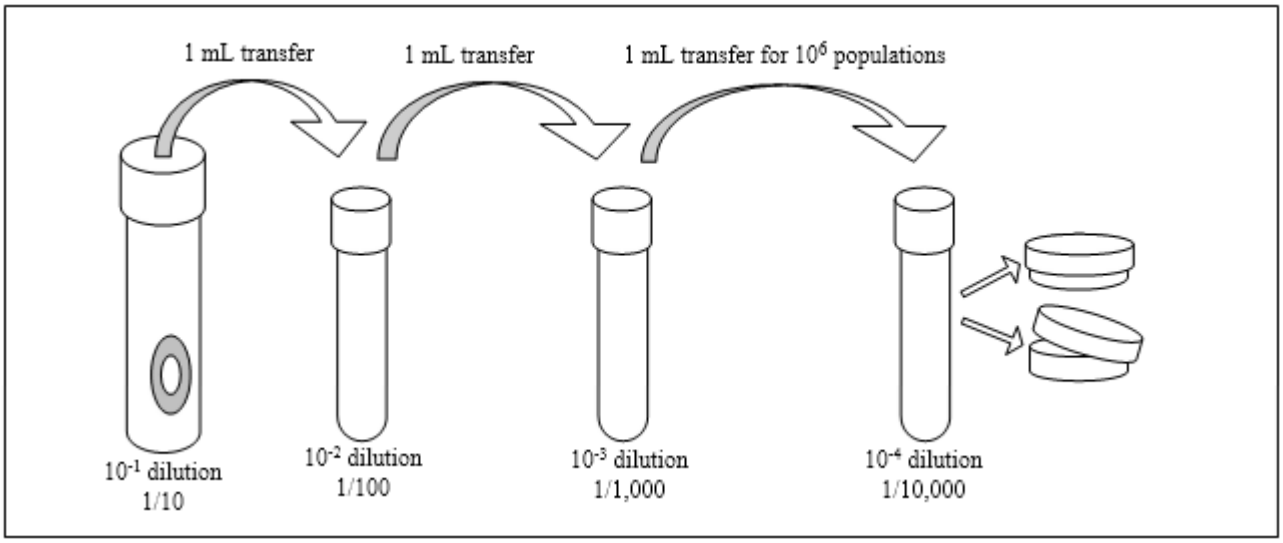
6. From each tube, transfer a 1mL aliquot to a dilution tube containing 9.0mL of sterile distilled water.

7. Vortex the tube for not less than 5 seconds.

8. Repeat as needed until the proper plating dilution is reached but do not plate.

Population Assay Instructions
Apex Products

9. Proceed to heat shock process.



10. In a preheated bath, heat-shock the tubes according to the test organism (see Table 1) starting the timing immediately upon insertion of sample into the preheated bath.

11. Remove tubes and cool rapidly in ice bath.

12. Plate each heat shock tube twice for a total of 8 plates.

13. Pour approximately 20 mL of melted growth medium cooled at 45° to 50°C 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.

14. Pour control plate.

15. Allow to solidify then invert and incubate plates according to test organism (see Table 1). If incubating at 55-60°C, putting the plates in a plastic bag will help avoid desiccation of agar.

16. 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, but not less than six per USP.

17. Average the counts and then multiply by the dilution factor.

18. Document all information.

OPTION 2:

Population Assay Instructions
Apex Products

1. Use one 5-mL pipette to transfer 5-mL of 0.1% Tween 80 into each 19.5 x 145-mm, flat-bottom tube (containing the four glass beads). Rinse Fluid D may be substituted for the 0.1% Tween 80.

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 stainless steel discs or ribbons from the lot to be assayed.

4. Place one stainless steel disc into each of the four flat-bottom tubes containing the 5 mL of 0.1% Tween 80.

5. Sonicate each tube for not less than (NLT) three minutes.

6. Vortex each tube for NLT five minutes.

7. Use the second 5-mL pipette to add 5-mL sterile purified water to each of the four 19.5 x 145-mm tubes Vortex for 30 seconds.

   NOTE: When adding volumes of sterile fluid (water, Tween 80 (0.1%) or Fluid D) to vortexed units in flat-bottomed tubes, be careful not to contaminate the tip of a pipette by touching it to a receiving tube.

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

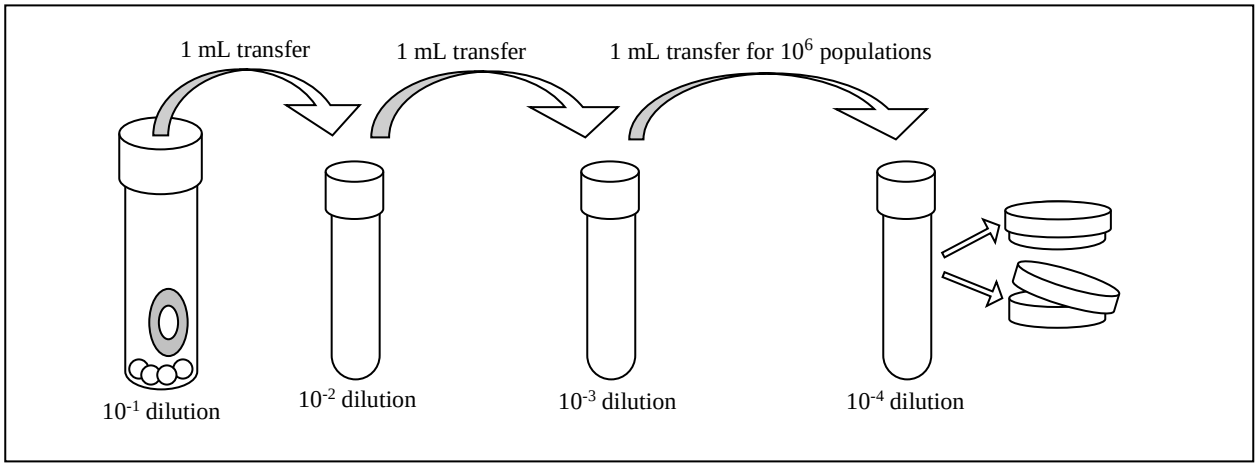
9. Remove tubes and cool rapidly in ice bath.

10. Dilution series for 10^6 spores per disc (modify, as appropriate, for spore populations higher/lower than 10^6 per disc):

    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 45º to 50ºC into the Petri plates. Swirl to ensure adequate mixing and allow the agar to solidify. Do not use agar

Population Assay Instructions
Apex Products

that has been melted and held longer than eight hours. Repeat the above dilution sequence for the remaining three heat-shocked tubes.



11. Pour control plate.

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

13. 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, but not less than six per USP.

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

15. Document all information.

Population Assay Instructions
Apex Products

Table 1. Heat-shock and Incubation Temperatures for Mesa Labs, Bozeman Manufacturing Facility 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'		
B. subtilis '6633'		
B. subtilis 'DSM4181'	95 - 100°C for 15 minutes	48 - 52°C for 48 hours
B. smithii	95 - 100°C for 15 minutes	48 - 52°C for 48 hours*
C. sporogenes	65 - 70°C for 20 minutes	35 - 39°C for 48 hours, anaerobic conditions
B. pumilus	65 - 70°C for 15 minutes	30 - 35°C for 48 hours
B. cereus		
B. megaterium		
B. licheniformis		

* Bag plates to avoid dehydration of media at this temperature.

** Start timing immediately upon insertion of sample into preheated bath.