

Population Assay Instructions DriAmp

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 four 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 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* (Water for Injection (WFI) is not recommended), 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 DriAmp. Tween 80 (0.1%) or Fluid D is needed for DriAmp.

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

Population Assay Instructions DriAmp

sterile Petri plate. The control plate should be incubated with the plates from the assay and should result in no growth.

III. DriAmp Population Assay (method for assaying 10^6 spores per indicator):

1. Use one 10 mL pipette to add 10.0 mL of 0.1% Tween 80 or Fluid D into each 19.5 x 145 mm flat-bottom tube.
2. Use one 10 mL pipette to add 9 mL of sterile purified water into each 16 x 125 mm dilution blank tube.
3. Randomly select four DriAmp indicators from the lot to be assayed.
4. With all sand in the bottom of the ampoule, snap the top of the ampoule as follows:

Figure 1



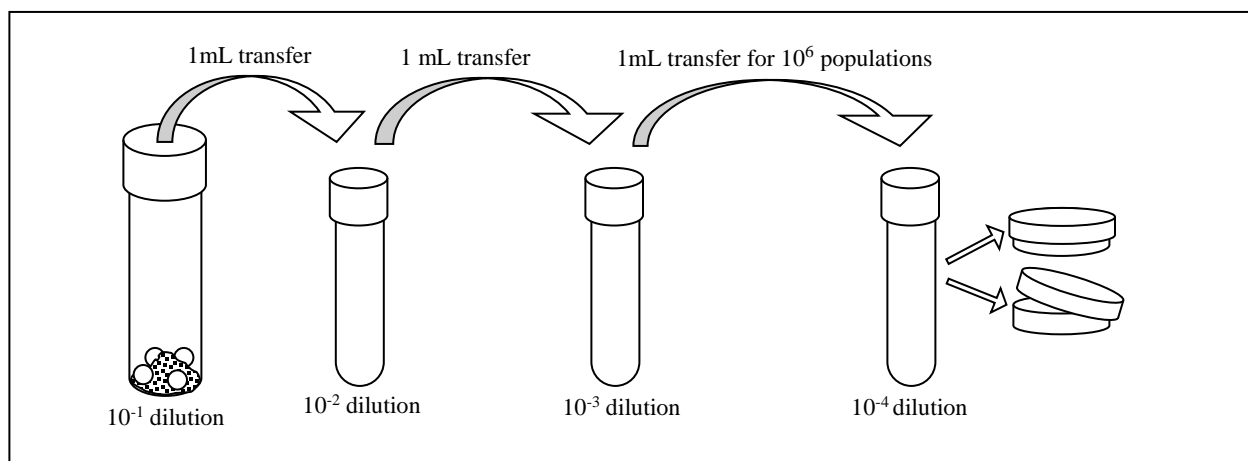
- 4.1 Safety goggles should be worn as a precaution.
- 4.2 Hold the body of the ampoule in one hand and the top of the ampoule in the other hand.
- 4.3 Position thumb tips spread away from the scored line of the ampoule. The first knuckle of each thumb should touch, acting as a hinge (see Figure 1).
NOTE: Laceration can occur if thumb tips are touching along the scored line.
- 4.4 Apply pressure to the scored line.
5. Pour the sand from each DriAmp ampoule into separate 19.5 x 145 mm flat-bottom tubes containing the four glass beads.
6. Sonicate each 19.5 x 145 mm tube for five minutes.
7. Vortex each tube for one minute.
8. In a pre-heated bath, heat-shock each 19.5 x 145 mm tube according to the test organism

Population Assay Instructions DriAmp

(see Table 1) starting the timing immediately upon insertion of the sample into the preheated bath.

- Remove tubes and cool rapidly in ice bath.
- Dilution series for a 10^6 spore population:

A dilution series will be made from each 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 2 mL pipette, transfer a 1 mL aliquot to a dilution blank containing 9 mL 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 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. Repeat the above dilution sequence for the remaining three heat-shocked tubes.



- Pour control plate.
- Allow to solidify then invert and incubate plates according to test organism (see Table 1).
- 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.
- Average the counts and then multiply by the dilution factor to calculate the population per original unit.

Population Assay Instructions DriAmp

15. Document all information.

Table 1. Heat-shock and Incubation Temperatures for Mesa Labs, Bozeman Manufacturing Facility Biological Indicator Test Organisms

Test Organism	Heat shock**	Incubation
<i>G. stearothermophilus</i>	95 – 100 °C for 15 minutes	55 – 60 °C for 48 hours*
<i>B. atrophaeus</i>	80 – 85 °C for 10 minutes	30 – 35 °C for 48 hours
<i>B. subtilis</i> '5230'		
<i>B. subtilis</i> '6633'		
<i>B. subtilis</i> 'DSM4181'	95 – 100 °C for 15 minutes	48 – 52 °C for 48 hours
<i>B. smithii</i>	95 – 100 °C for 15 minutes	48 – 52 °C for 48 hours*
<i>C. sporogenes</i>	65 – 70 °C for 20 minutes	35 – 39 °C for 48 hours, anaerobic conditions
<i>B. pumilus</i>	65 – 70 °C for 15 minutes	30 – 35 °C for 48 hours
<i>B. cereus</i>		
<i>B. megaterium</i>		
<i>B. licheniformis</i>		

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

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