Population Assay Instructions

SterilAmp® II, SterilAmp® II 5230, MagnaAmp®

I. List of Components:

Mesa Labs offers components for performing a population assay. See the part numbers below and the items each includes.

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

Required Items:

- Growth medium
- 160 mL purified sterile water*
- Sterile flat-bottom tubes, 19.5 x 145 mm
- Sterile dilution blank tubes, 16 x 125 mm
- Pipettes: 10, 5, 2, and 1 mL
- Sterile stainless-steel rod or forceps
- Graduated cylinder
- Sterile 250 mL Pyrex® bottle
- Petri plates, 15 x 100 mm
- Timing device
- Device for melting growth medium
- Instrument for tempering growth medium
- Vortex machine
- Ultrasonic cleaner (45-60 kHz)
- Heat-shock bath
- Ice bath
- Incubator
- Safety goggles

*Throughout this procedure when sterile purified water is referenced, this includes sterile distilled, deionized (DI) or reverse osmosis (RO) water. Water for Injection (WFI), phosphate buffers, or physiological saline solutions are not recommended.

II. Preparing the Growth Medium for use:

NOTE: If you 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. Temper the agar to 45 - 50°C until ready to use.

3. Do not use agar that has been melted and held for longer than eight hours.

4. A control plate should be poured with each assay to verify the sterility of the growth medium. The control plate should be incubated with the plates from the assay and should result in no growth.

III. Procedure:

1. Use one 10 mL pipette to transfer 9 mL of sterile purified water into four (for 10^5 BI) or six (for 10^6 BI) 16 x 125 mm dilution blank tubes.

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

3. Place all four ampoules into a sterile 250 mL Pyrex bottle. Crush the ampoules to shards using either a sterile stainless-steel rod or sterile forceps (safety goggles should be worn as a precaution).

4. Add the appropriate product specific amount of sterile purified water (see below) to the 250 mL Pyrex bottle, rinsing the crushing device with the water as it is added.

   4.1 SterilAmp II and SterilAmp II 5230:

   - Fill volume is 0.3 mL per ampoule.
   - There are four ampoules for a total of 1.2 mL.
   - Add 98.8 mL of water to bring the total volume to 100 mL.

   4.2 18-mm SterilAmp II:

   - Fill volume is 0.13 mL per ampoule.
   - There are four ampoules for a total of 0.52 mL.
   - Add 99.48-mL of water to bring the total volume to 100 mL.

   4.3 MagnaAmp:

   - Fill volume is 1.2 mL per ampoule.
   - There are four ampoules for a total of 4.8 mL.
   - Add 95.2-mL of water to bring the total volume to 100 mL.

5. Vortex the sample for no less than one minute.

6. Allow the Pyrex container to sit for five minutes to allow air bubbles to dissipate.

7. Sonicate the sample for five minutes using 45 – 60 kHz.

8. If using Mesa's PAK-G Population Assay Kit, remove the beads from one 19.5 x 145 mm flat bottomed tube.

9. Vortex sample again for approximately 10 seconds making sure a thorough vortex is achieved, especially with MagnaAmp.

10. Use the second 10 mL pipette to transfer a 10 mL aliquot from the Pyrex bottle into the 19.5 x 145 mm flat bottom tube.

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

12. At the end of the time, remove the tube and cool rapidly in an ice bath.

13. Two dilution series will be made from the heat-shocked tube.
NOTE: It is extremely important to make each serial transfer immediately after vortexing.

14. Dilution series for a 10^5 and 10^6 population biological indicator (BI):

    14.1 Vortex the heat-shocked tube for at least 10 seconds, then use a 1 mL pipette to transfer a 1 mL aliquot to a dilution blank containing 9 mL of sterile purified water.

    14.2 Vortex the dilution tube for at least 10 seconds, then use a 1 mL pipette to transfer 1 mL to a second dilution blank containing 9 mL of sterile purified water.

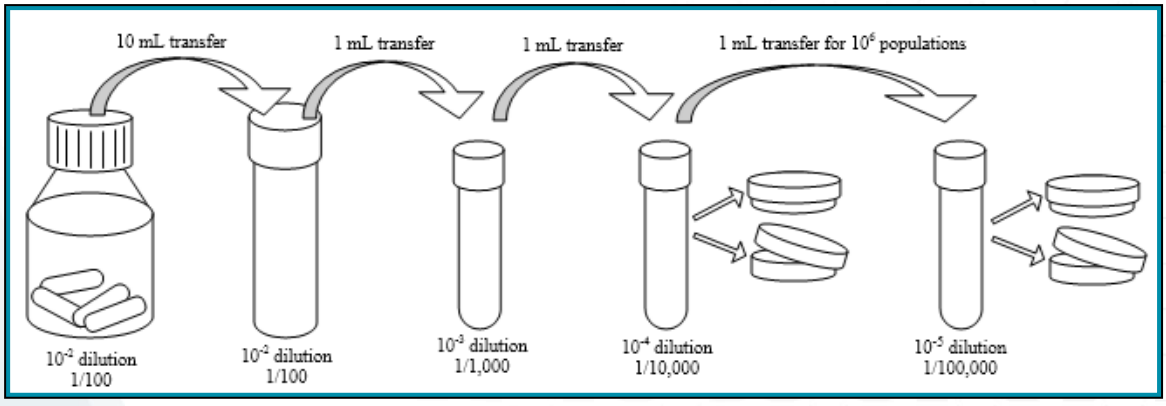
    14.3 For a 10^5 BI, proceed to step 14.5.

    14.4 For a 10^6 BI, Vortex the dilution tube for at least 10 seconds, then use a 1 mL pipette to transfer 1 mL to a third dilution blank containing 9 mL of sterile purified water.

    14.5 Vortex the dilution tube for at least 10 seconds, then use a 2 mL pipette to withdraw 2 mL. Dispense 1 mL into each of two 15 x 100 mm Petri plates.

    14.6 Repeat steps 14.1 through 14.4 once more.

15. Pour approximately 20 mL of melted growth medium tempered to 45 - 50°C into the Petri plates and



swirl to ensure adequate mixing. Allow the agar to solidify.

16. Pour a control plate.

17. Once the agar is solidified, invert the plates and incubate according to the test organism (see Table 1).

18. After not less than (NLT) 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.

19. Average the counts and then multiply by the inverse of the dilution factor. This value must then be divided by four to calculate the population per original unit.

20. Document all information.

Table 1: Heat-shock and Incubation Temperatures

Test Organism	Heat shock*	Incubation (NLT 48 hours)
G. stearothermophilus	95 - 100°C for 15 minutes	55 - 60°C**
B. subtilis '5230'	80 - 85°C for 10 minutes	30 - 35°C

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

Reference Document:

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

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