

Population Assay Instructions SterilAmp II, SterilAmp II “5230” and MagnaAmp

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 6 mm 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 containing 240 mL of sterile Difco brand growth medium

Items required are: growth medium, sterile flat-bottom tubes with four 6mm beads, sterile blank tubes 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 SterilAmp II and MagnaAmp. Additionally, a sterile stainless steel rod or sterile forceps are needed for the SterilAmp II and MagnaAmp assays. A sterile 250 mL Pyrex bottle and graduated cylinder will be needed when performing assays for SterilAmp and MagnaAmp.

*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.

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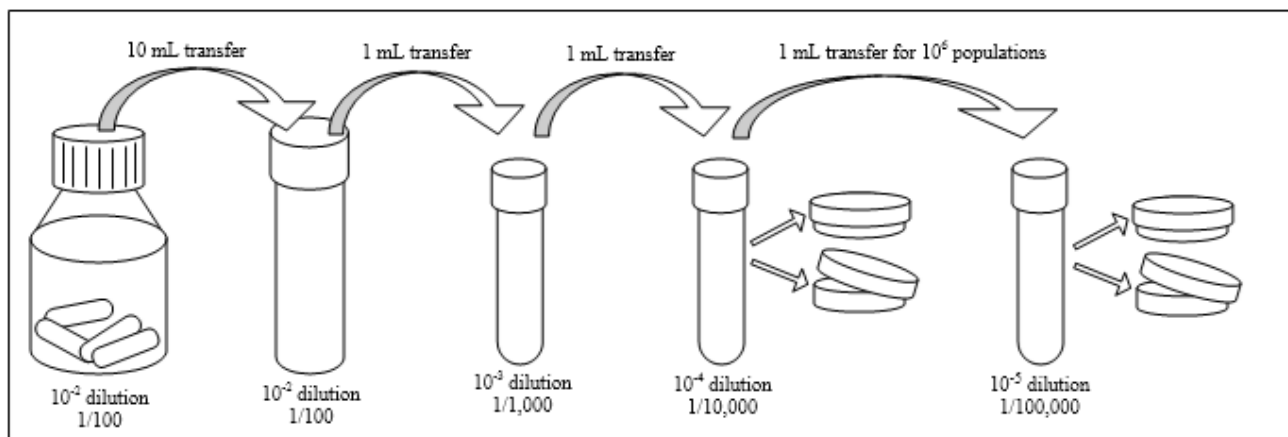
III. SterilAmp II, SterilAmp II “5230” and MagnaAmp Population Assay (method for assaying liquid carriers):

1. Use one 10 mL pipette to transfer 9 mL of sterile purified water into six of the 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 (not included in kit). Crush the ampoules to shards using either a sterile stainless steel rod or sterile forceps.
4. Rinse the crushing device with the sterile purified water as it is added to the 250 mL Pyrex.
5. SterilAmp II and SterilAmp II “5230”:
 - 5.1 Fill volume is 0.3 mL per ampoule.
 - 5.2 There are four ampoules for a total of 1.2 mL.
 - 5.3 Add 98.8 mL of water to bring the total volume to 100 mL.
6. 18-mm SterilAmp II:
 - 6.1 Fill volume is 0.13 mL per ampoule.
 - 6.2 There are four ampoules for a total of 0.52 mL.
 - 6.3 Add 99.48-mL of water to bring the total volume to 100 mL.
7. MagnaAmp:
 - 7.1 Fill volume is 1.2 mL per ampoule.
 - 7.2 There are four ampoules for a total of 4.8 mL.
 - 7.3 Add 95.2-mL of water to bring the total volume to 100 mL.
8. Vortex sample for no less than one minute.
9. Allow Pyrex container to sit for five minutes to allow air bubbles to dissipate.
10. Sonicate the sample for five minutes using 45 – 60 kHz.
11. Remove the beads from one 19.5 x 145 mm flat bottomed tube.
12. Vortex sample again for approximately 10 seconds.

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- Use the second 10 mL pipette to transfer a 10 mL aliquot from the Pyrex bottle into the 19.5 x 145 mm flat bottomed tube.
- 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.
- Remove tube and cool rapidly in ice bath.
- 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. 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. From the heat-shocked tube, repeat the above dilution sequence one additional time.



- 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.

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20. Average the counts and then multiply by the dilution factor. This value must then be divided by four to calculate the population per original unit.
21. 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.