Population Assay Instructions

EZTest® H_2O_2 and Stainless-Steel Discs

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 with four 6 mm beads
- Sterile dilution blank tubes
- Pipettes: 10, 5, 2, and 1 mL
- 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

*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 for use.

3. A control plate should be poured with each assay 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

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medium into a sterile Petri plate. 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 add 10 mL of sterile purified water to each of four 19.5 x 145 mm flat bottom tubes with glass beads.

2. Use one 10 mL pipette to add 9 mL of sterile purified water into each of twelve 16 x 125 mm dilution blank tubes.

3. Randomly select four inoculated carriers from the lot to be assayed. See Appendix 1 for how to remove the inoculated carrier from EZTest H_2O_2.

4. Place one carrier per 19.5 x 145 mm flat bottom tube.

5. Sonicate the tubes with the carrier for 16 minutes.

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

   5.2 The rack should be all metal, preferably stainless steel.

   5.3 Tubes should be equidistant from each other.

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

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

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

7. At the end of the time, remove the tubes and cool rapidly in an ice bath.

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

9. Dilution series for a 10^6 population carrier:

   9.1 Vortex the heat-shocked tube for at least 10 seconds, then use a 2 mL pipette to transfer a 1 mL aliquot to a dilution blank containing 9 mL of sterile purified water.

   9.2 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.

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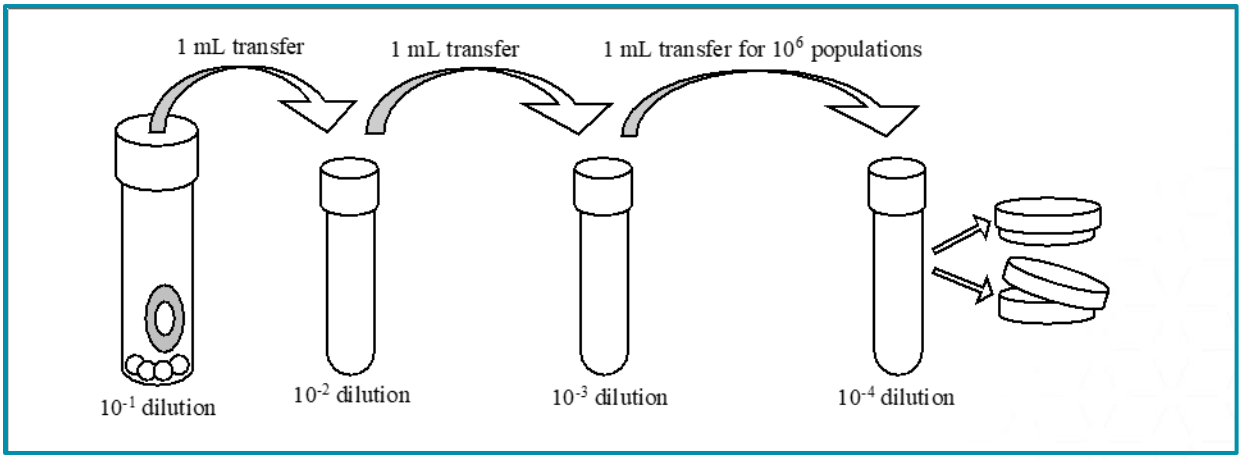
9.3 Vortex the dilution tube for at least 10 seconds, then use a 1 mL pipette to transfer 1 mL to a third dilution blank containing 9 mL of sterile purified water.

9.4 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.

9.5 Repeat steps 9.1 through 9.4 for the remaining heat-shocked tubes.



12. Pour approximately 20 mL of melted growth medium tempered to 45° to 50°C into the Petri plates and swirl to ensure adequate mixing. Allow the agar to solidify.

13. Pour control plate.

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

15. 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.

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

17. 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. pumilus	65 - 70°C for 15 minutes	30 - 35°C

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

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Appendix 1: Removing the Stainless-Steel 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 sterilized and discarded; do not attempt to assay the stainless-steel 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 as it may be 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. Begin tilting the EZTest to allow the media ampoule to slide out for easy removal. Care should be taken that the disc does not slide out as well.
5. Use sterile forceps to extract the inoculated stainless-steel carrier from the EZTest or pour the disc into the flat bottom tube.

Reference Document:

LP-306 Population Assay on Non-Cellulose Carriers (Based on)

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