POPULATION ASSAY KIT

INSTRUCTIONS FOR USE

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INSTRUCTIONS FOR USE

The following procedures have been provided with this kit and should be followed exactly when performing a population assay on Mesa Labs, Bozeman Manufacturing Facility biological indicators. There are nine sections to this document:

- List of components
- Preparing the growth medium for use
- Method for assaying inoculated paper carriers (EZTest®, MesaStrip)
- Method for assaying liquid carriers (SterilAmp®, MagnaAmp®)
- Method for assaying spore suspensions
- Method for assaying inoculated stainless steel discs
- Method for assaying DriAmp™
- Method for assaying SterilFlex™
- Method for assaying Apex H_2O_2 biological indicators

I. List of Components:

Quantity	Description
1	250-mL Wheaton bottle containing 240-mL growth medium
4	19.5 x 145-mm, sterile, flat-bottom tube with four 6-mm glass beads
4	19.5 x 145-mm, sterile, flat-bottom tube (for Apex BI) no glass beads
12	16 x 125-mm, sterile, dilution blank tube
8	1-mL pipettes
8	2-mL pipettes
2	5-mL pipettes
2	10-mL pipettes

Items required, but not included in the kit, are 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. An ultrasonic cleaner (45-60 kHz) is required when assaying SterilAmp, MagnaAmp, spore suspensions, stainless steel discs, DriAmp, Apex BIs and SterilFlex. Additionally, a sterile stainless steel rod or sterile forceps are needed for the SterilAmp and MagnaAmp assays. A sterile 250 mL Pyrex bottle and graduated cylinder will be needed when performing assays for SterilAmp, MagnaAmp and SterilFlex. Tween 80 (0.1%) or Fluid D is needed for stainless steel discs (not Apex BI), DriAmp and SterilFlex. A calibrated pipette will be needed for spore suspension assays. Finally, a sterile hemostat, scissors, and stir bar along with a stir plate are needed for SterilFlex assays.

NOTE: For each transfer of liquid it is important to rinse the pipette. After each transfer into a 9-mL water blank, re-pipette 1-mL of liquid into the pipette and again dispense in to the same tube. Rinsing will ensure any remaining spores in the pipette will be recovered; failure to do this may result in a low recovery.

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.

II. Preparing the Growth Medium for use:

NOTE: The medium was prepared at Mesa Labs, Bozeman Manufacturing Facility 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 stored at 45° to 50°C until ready for use.

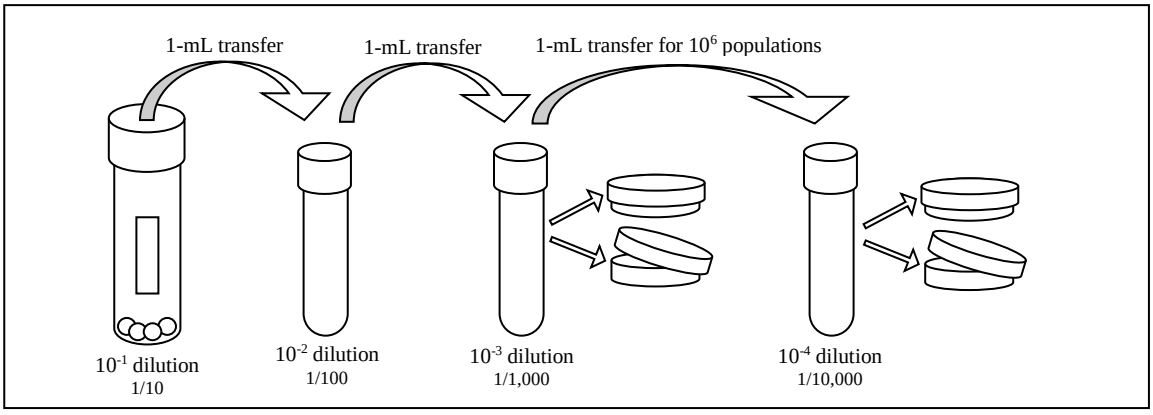
3. A control plate should be poured with each assay. The purpose of the control plate is to verify the sterility of the growth medium. The control plate should be prepared upon completion of the assay and it consists of pouring the remaining growth medium into a sterile Petri plate. The control plate should be incubated with the plates from the assay and should result in no growth.

III. EZTest and MesaStrip Population Assay (method for assaying inoculated paper carriers):

NOTE: To avoid inaccurate colony counts, it is important to perform the initial transfer using the 2-mL pipette as this pipette has the largest bore size. This will help avoid clogging the pipette tip with fibers.

1. Use one 5-mL pipette to transfer 5-mL of sterile purified water into each 19.5 x 145-mm, flat-bottom tube (containing the four glass beads).
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 paper carriers from the lot to be assayed. (See Appendix 1 for instructions on removing paper carrier from EZTest.)
4. Place each carrier into a screw cap 19.5 x 145-mm, flat-bottom tube.
5. Vortex until the paper carrier is macerated to pulp, about four to seven minutes.
6. Use the second 5-mL pipette to add an additional 5-mL of sterile purified water to each macerated strip. Vortex for 30 seconds.
7. 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 sample into the pre-heated bath.
8. Remove tubes and cool rapidly in ice bath.
9. Dilution series for a 10^5 and 10^6 population:

   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 and rinse pipette. 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 and rinse after each transfer). 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 50ºC 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.



10. Pour control plate.
11. Allow to solidify then invert and incubate plates according to test organism (see Table 1).
12. 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.
13. Average the counts and then multiply by the dilution factor to calculate the population per original unit.
14. 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
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. subtilus '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		

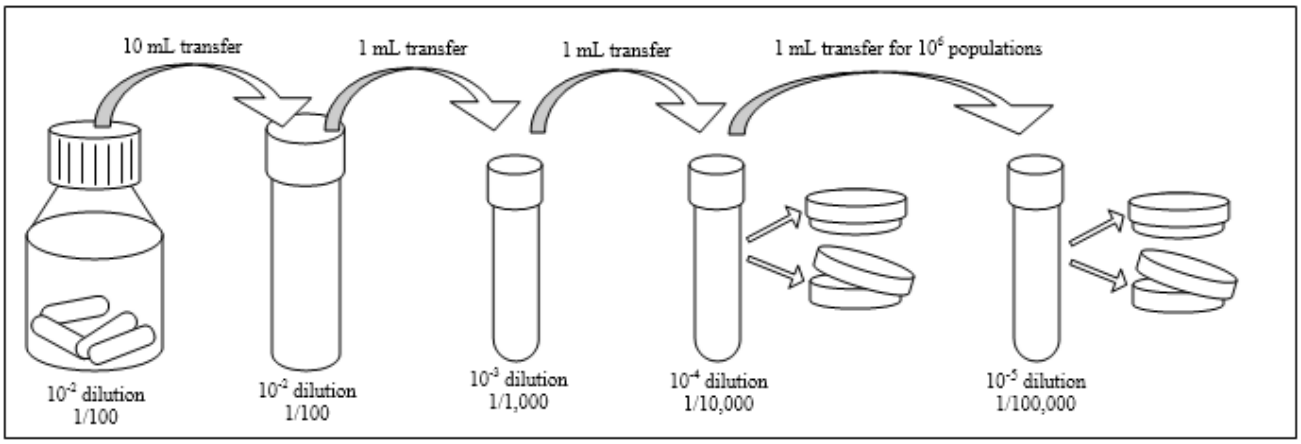
* Bag plates to avoid dehydration of media at this temperature.
** Start timing immediately upon insertion of sample into preheated bath.

IV. SterilAmp 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 and SterilAmp "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:
   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. Prior to sonication allow Pyrex container to sit for five minutes to allow air bubbles to dissipate.
10. Sonicate the sample for five minutes.
11. Vortex the sample for not less than one minute.
12. Remove the beads from one 19.5 x 145 mm flat-bottomed tube.
13. Use the second 10-mL pipette to transfer a 10-mL aliquot from the Pyrex bottle into the tube.
14. 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.
15. Remove tube and cool rapidly in ice bath.
16. Dilution Series for a 10^5 and 10^6 population:

    <u>Two</u> 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 and rinse the pipette. 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, rinsing pipette after each transfer)**. 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 50ºC 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.



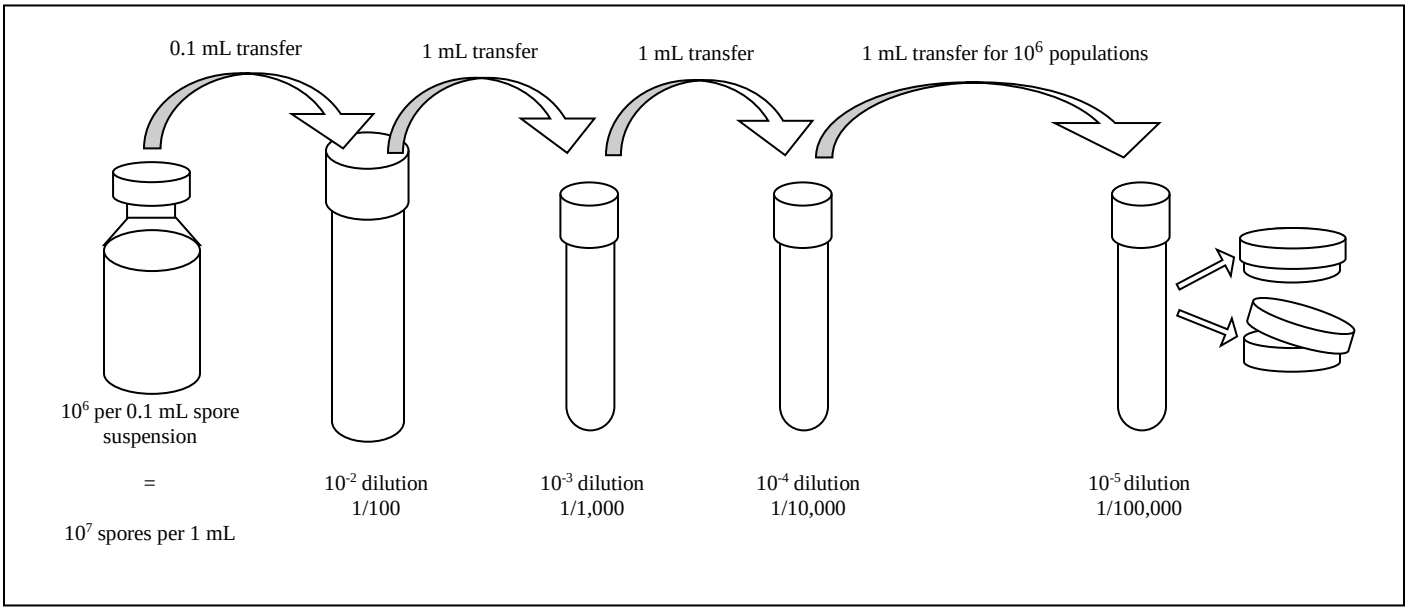
17. Pour control plate.
18. Allow to solidify then invert and incubate plates according to test organism (see Table 1).
19. After 48 hours of incubation, remove the plates from the incubator and count the colony forming units (CFU) on each plate. Preferably plates with counts between 30 and 300 CFU should be used, but not less than six per USP.
20. Average the counts and then multiply by the dilution factor. <u>This value must then be divided by four to calculate the population per original unit.</u>
21. Document all information.

V. Spore Suspension Population Assay (method for assaying 10^6 spores per 0.1 mL):

1. Remove the beads from one 19.5 x 145 mm tube. Use a 10-mL pipette to transfer 9.9-mL of sterile purified water into the 19.5 x 145-mm tube.
2. Use a 10-mL pipette to transfer 9 mL of sterile purified water into six of the 16 x 125-mm dilution blank tubes.
3. Vortex the spore suspension vial for not less than one minute and let stand for five minutes until all bubbles disappear.
4. Sonicate the spore suspension vial for three to five minutes and check microscopically for clumping. Repeat this step until clumps are dispersed.
5. Vortex spore suspension vial for at least 10 seconds
6. Use a calibrated pipette to extract and transfer 0.1-mL from the spore suspension vial and add it to the 19.5 x 145-mm tube containing the 9.9-mL sterile purified water and rinse the pipette. Vortex this tube for 30 seconds.
7. 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.
8. Remove tube and cool rapidly in ice bath.
9. Dilution series for a 10^6 population (modify as appropriate for suspensions with higher/lower concentrations of spores per 0.1-mL).

   <u>Two</u> 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 and rinse the pipette. 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 per 0.1-mL population rinsing the pipette after each transfer.** 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 50ºC 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.



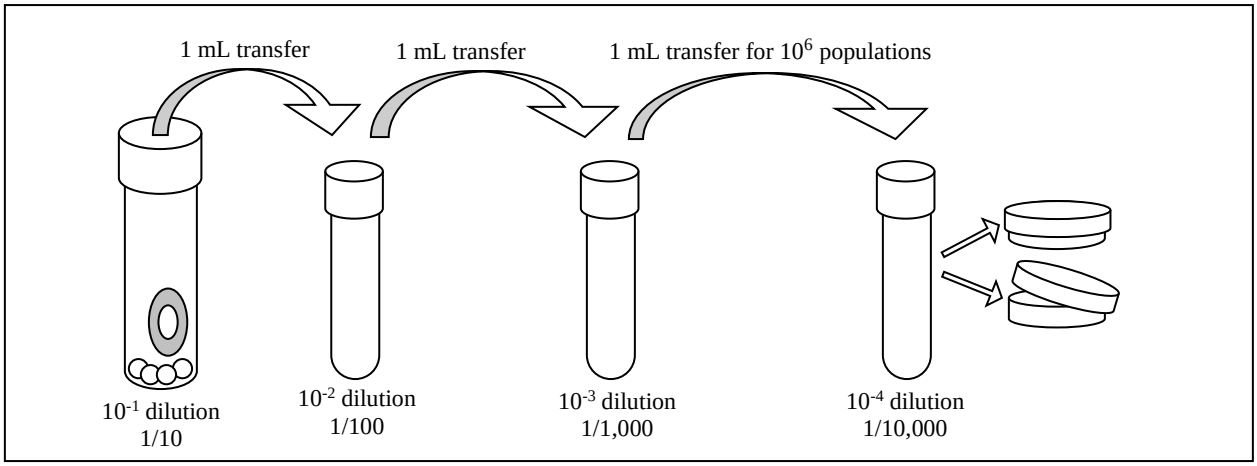
10. Pour control plate
11. Allow to solidify then invert and incubate plates according to test organism (see Table 1).
12. After 48 hours of incubation, remove the plates from the incubator and count the colony forming units (CFU) on each plate. Preferably plates with counts between 30 and 300 CFU should be used, but not less than six per USP.
13. Average the counts and then multiply by the dilution factor. The result is the concentration of the spores per 1-mL. Divide this by 10 to obtain the concentration of spores per 0.1-mL for comparison to the label claim.
14. Document all information.

VI. Stainless Steel Disc Population Assay (method for assaying 10^6 spores per disc) – NOT Apex BI:

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 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 three to five minutes.
6. Vortex each tube for 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.
8. Vortex each tube for five minutes.
9. 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.
10. Remove tubes and cool rapidly in ice bath.
11. 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 and rinse pipette. 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 and rinse after each transfer.** 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 mediumcooled to 45º to 50ºC 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.

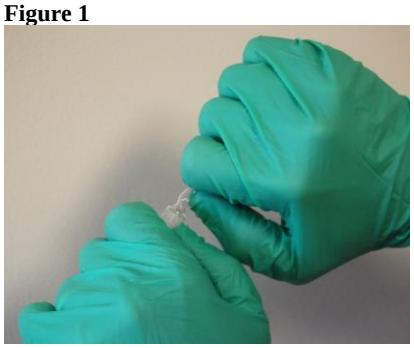


12. Pour control plate.
13. Allow to solidify then invert and incubate plates according to test organism (see Table 1).
14. After 48 hours of incubation, remove the plates from the incubator and count the colony forming units (CFU) on each plate. Preferably plates with counts between 30 and 300 CFU should be used, but not less than six per USP.
15. Average the counts and then multiply by the dilution factor to calculate the population per original unit.
16. Document all information.

VII. 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:

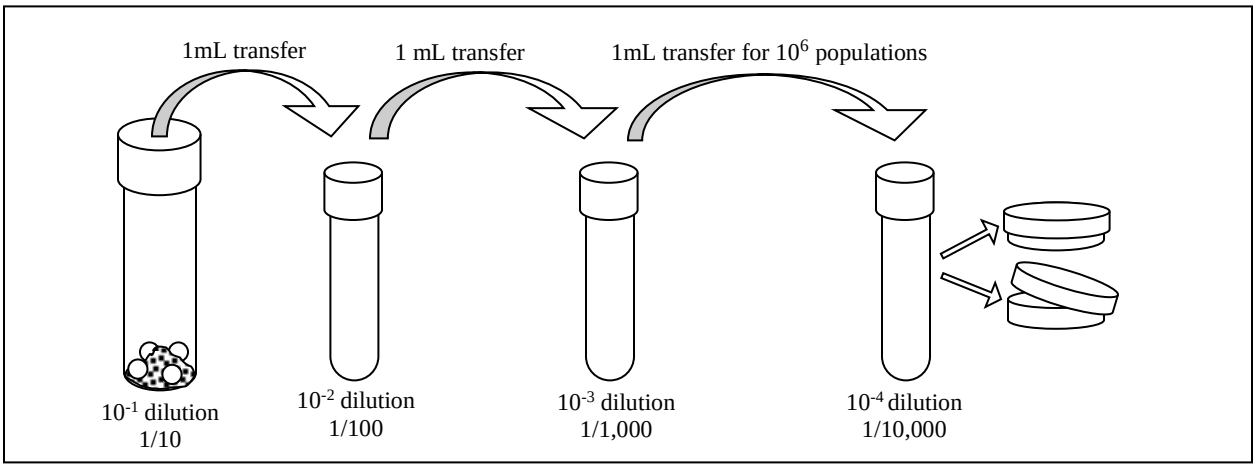
Figure 1



i. Safety goggles should be worn as a precaution.
ii. Hold the body of the ampoule in one hand and the top of the ampoule in the other hand.
iii. 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.
iv. 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 (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 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 and rinse pipette. 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 and rinse after each transfer.** 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 that has been melted and held longer than eight hours. Repeat the above dilution sequence for the remaining three heat-shocked tubes.



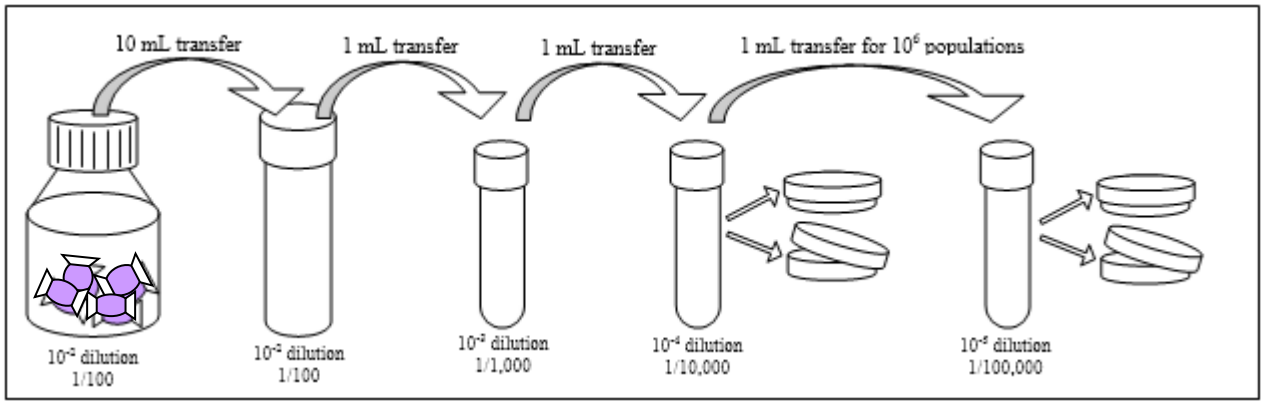
11. Pour control plate.
12. Allow to solidify then invert and incubate plates according to test organism (see Table 1).
13. After 48 hours of incubation, remove the plates from the incubator and count the colony forming units (CFU) on each plate. Preferably plates with counts between 30 and 300 CFU should 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.

VIII. SterilFlex Population Assay:

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 SterilFlex units from the lot to be assayed.
3. Use a hemostat to grip the corner of one SterilFlex unit. Hold the unit over a sterile 250-mL Pyrex bottle and cut the adjacent corner with sterile scissors. NOTE: There is pressure in the unit and once cut, it may squirt liquid.
4. Allow the contents of the SterilFlex to drip into the Pyrex bottle.
5. Cut the remaining part of the SterilFlex into small sections allowing them to fall into the Pyrex bottle.
6. Repeat steps 3 – 5 with the remaining three SterilFlex units.
7. Add a sterile stir bar to the Pyrex bottle.
8. Add 99.8-mL of 0.1% Tween 80 or Fluid D to the Pyrex bottle. Be sure to rinse the scissors with the 0.1% Tween 80 or Fluid D as it is added to the Pyrex bottle.

9. Sonicate the sample for three minutes.
10. Vortex the sample for one minute.
11. Place on stir plate for 10 minutes and stir rapidly.
12. Remove the beads from one 19.5 x 145-mm flat-bottomed tube.
13. Use the second 10-mL pipette to transfer a 10-mL aliquot from the Pyrex bottle into the tube.

14. In a preheated 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.
15. Remove tube and cool rapidly in ice bath.
16. Dilution Series for a 10^6 population:



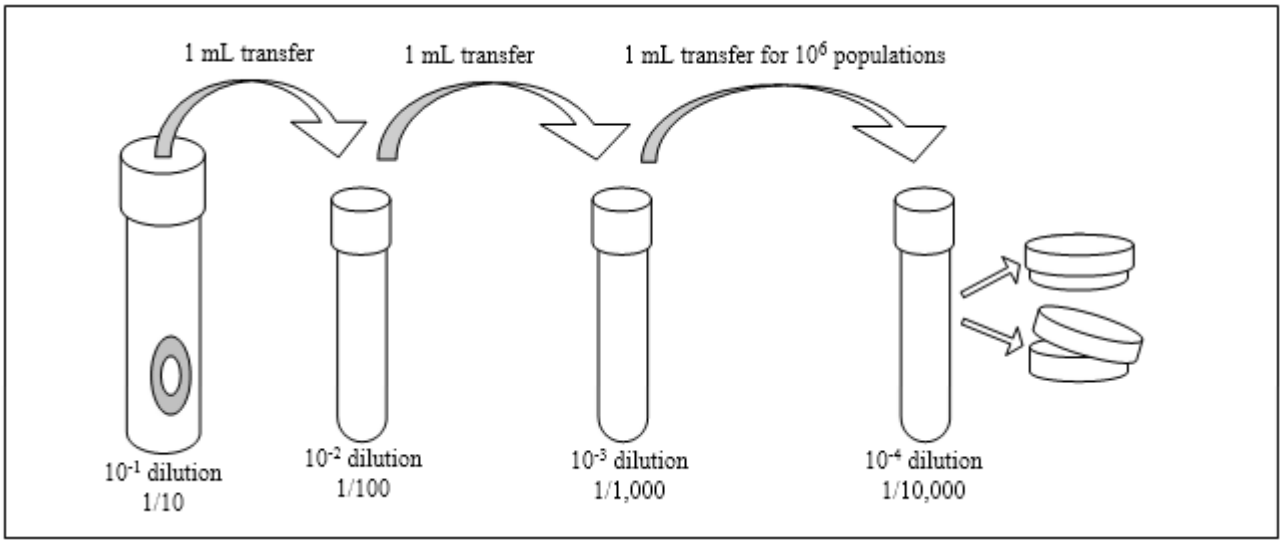
Two dilution series will be made from the heat-shocked tube. NOTE: It is extremely important to make each serial transfer immediately after vortexing. Vortex the heat-shocked tube for at least 10 seconds. Using a 1-mL pipette, transfer a 1-mL aliquot to a dilution blank containing 9-mL of sterile purified water and rinse the pipette. 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 rinsing the pipette after each transfer.** 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 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. From the heat-shocked tube, repeat the above dilution sequence one additional time.

17. Pour control plate.
18. Allow to solidify then invert and incubate plates according to test organism (see Table 1).
19. 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.
20. Average the counts and then multiply by the dilution factor. <u>This value must then be divided by four to calculate the population per individual SterilFlex unit.</u>
21. Document all information.

VIV. Apex Biological Indicators (BI) Population Assay:

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.
2. Do not place more than one BI in each tube.
3. Sonicate not less than 15 minutes.
   - If using a sonicator with a holding rack, ensure it is metal.
   - Tubes should be equidistant from each other
   - Ensure the rack in the sonic bath is suspended above the bottom of the bath, so that sonic energy reaches the test tubes uniformly
   - 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.
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.

Appendix 1

Removing the Paper 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 killed and discarded; one should not attempt to assay the paper 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; one may find 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. The glass media ampoule should easily fall out of the EZTest when turned upside down. If not, gently tap the open end of the EZTest on the bench top to aid removal of the glass media ampoule.
5. Use sterile forceps to extract the inoculated paper carrier from the EZTest.