

Instructions for Performing Population Assay of SterilAmp® Biological Indicator

Step 1: In order to verify a population of spores in a glass ampoule, the spores must first be recovered from the ampoule. To begin, randomly select four (4) ampoules from the lot to be assayed.

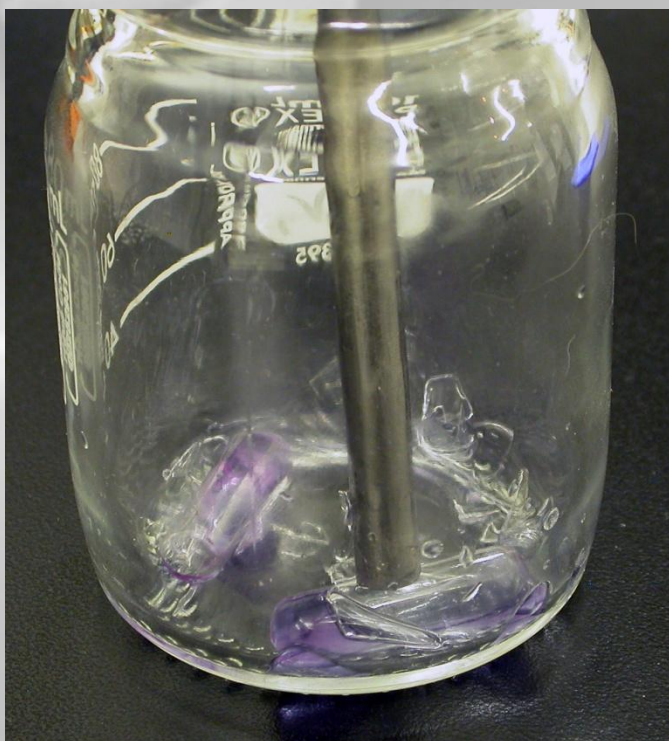


Step 2: Place four (4) ampoules in a sterile, 100 ml Pyrex bottle.



Stainless Steel Rod

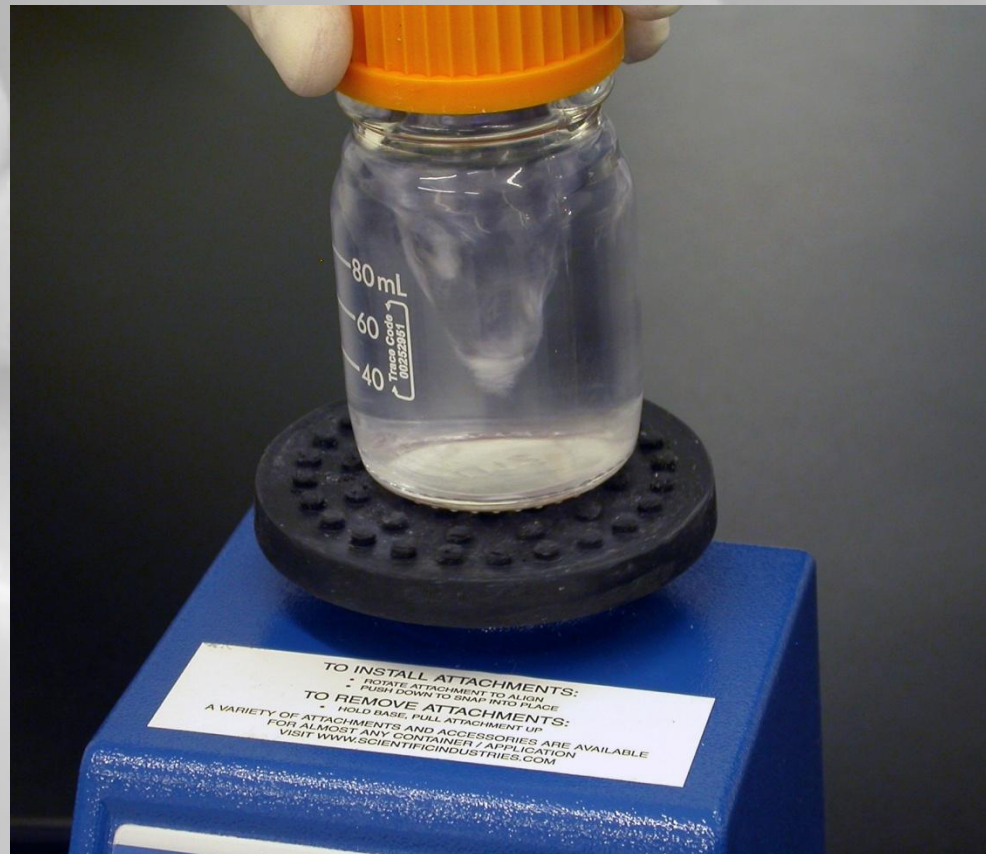
Step 3: Using a sterile stainless steel rod or sterile forcep, crush the ampoules to shards. Ensure the bottle is sitting flat on the bench when striking the ampoule.



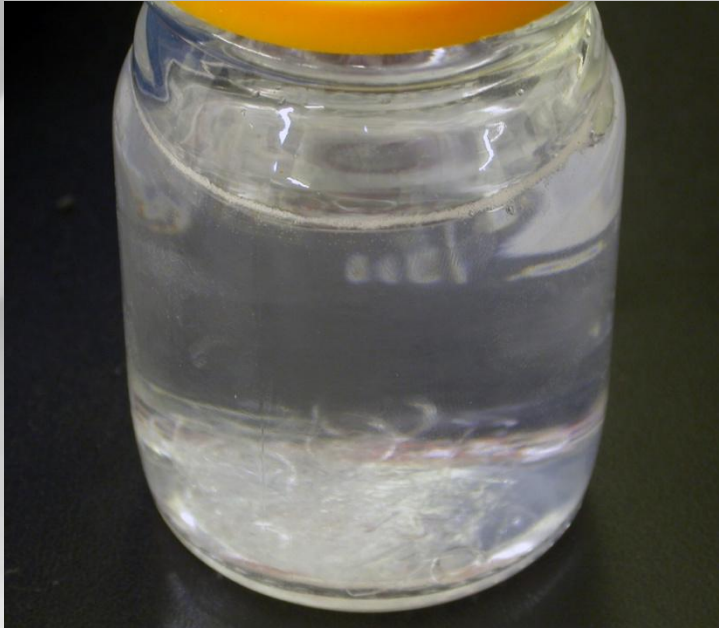
Step 4: Rinse the crushing device with 98.8 mls sterile purified water as it is added to the 100 ml bottle.



Step 5: Vortex the bottle for at least one minute to achieve a homogeneous blend.



Step 6: Allow the bottle to stand for five (5) minutes after vortexing to allow air bubbles to dissipate before sonicating. Sonicate the sample for 3-5 minutes at 46 to 60 kHz.



Immediately after vortexing



Standing time of 5 minutes

Step 7: Vortex sample again for approximately 10 seconds. Immediately after vortexing, pipette 10.0 mls from the bottle into a sterile, screw cap 19.5 x 145 mm flat-bottomed tube to be heat shocked.

Step 8: Heat shock the 19.5 x 145 mm flat bottom tube containing the 10.0 mls of the dilution in a water or glycol bath preheated to the required temperature for the appropriate length of time. Remove tube and cool in an ice bath (0° to 4° C) for two (2) minutes.

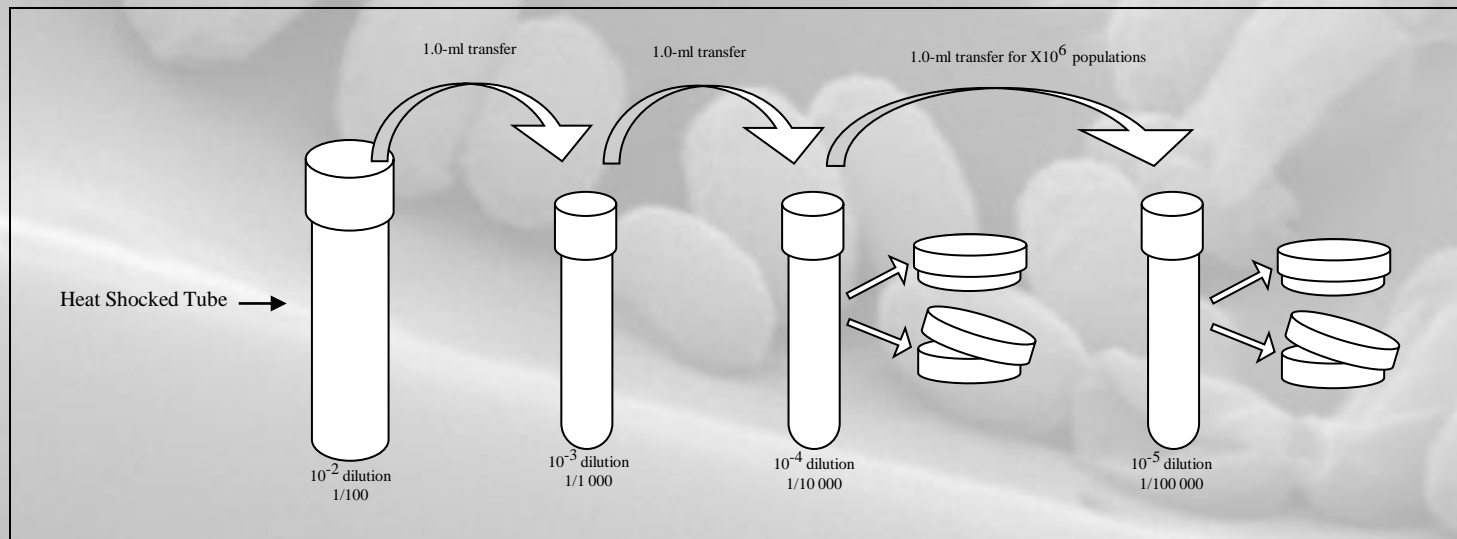
Species	Heatshock Temp.	Heatshock Time
<i>G. stearothermophilus</i>	95 - 100°C	15 minutes
<i>B. subtilis</i> “5230”	65 - 70°C	15 minutes

Two dilution series will be made from the heatshocked tube. Vortex the heat-shocked tube at least 10 seconds before a transfer is made. This will avoid settling of the suspension.

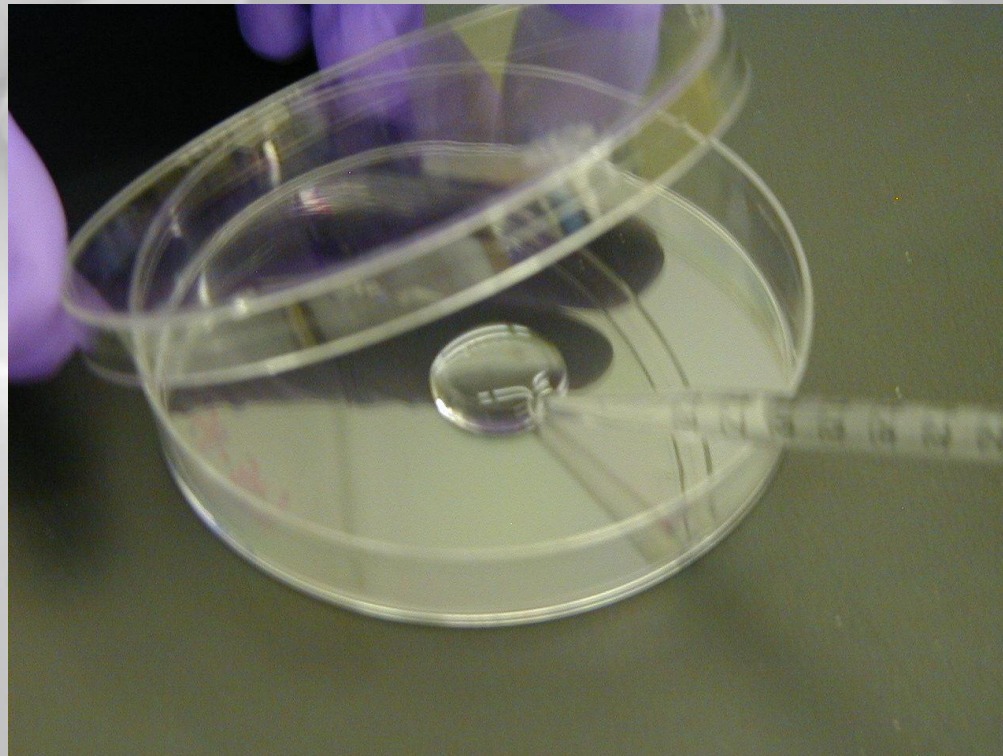
Step 9: Transfer two (2) 1.0 ml aliquots to two (2) 16 x 125 mm dilution blank tubes, each containing 9.0 ml of sterile purified water.

Step 10: Vortex each dilution blank tube for at least ten (10) seconds. Transfer 1.0 ml from each dilution blank tube to a second dilution blank tube containing 9.0 ml of sterile purified water.

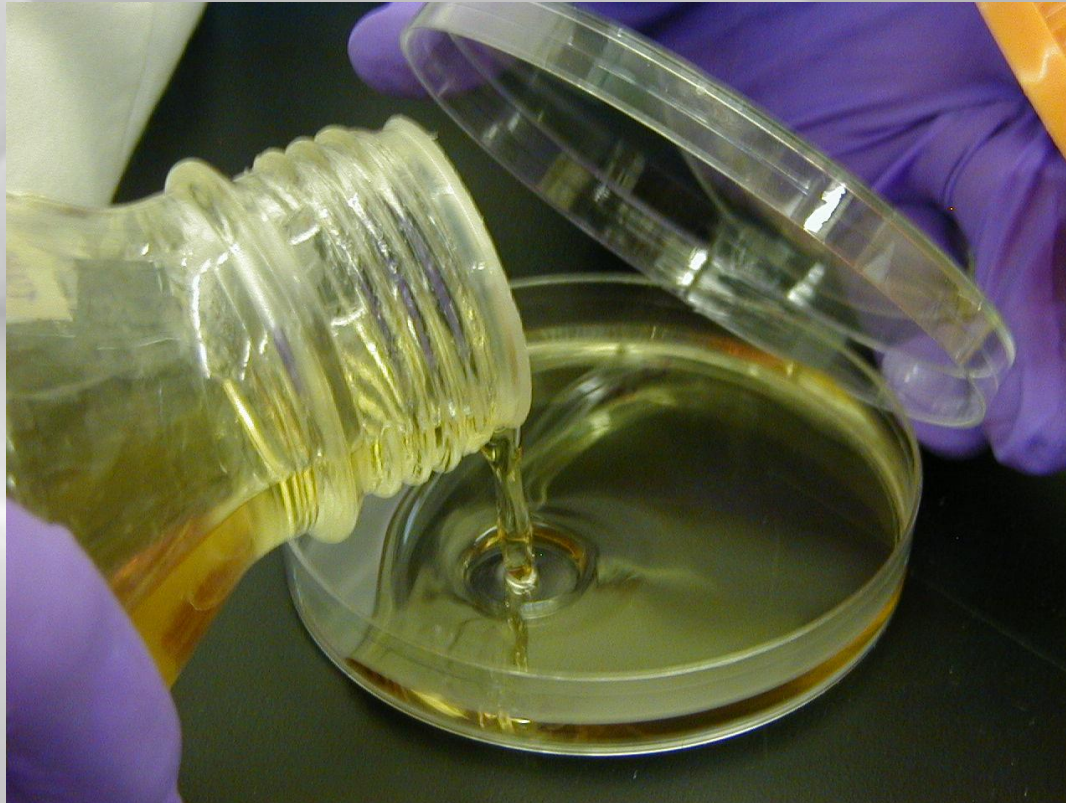
If a 10^6 population is present repeat this step one more time.



Step 11: Vortex each of the last tubes of the dilution series for ten (10) seconds. Pipette 1.0 ml into a 15 x 100 mm petri plate. Repeat this procedure to achieve two (2) replicate petri plates from each of the last tubes of the dilution series for a total of four (4) plates. Vortex before pipetting.



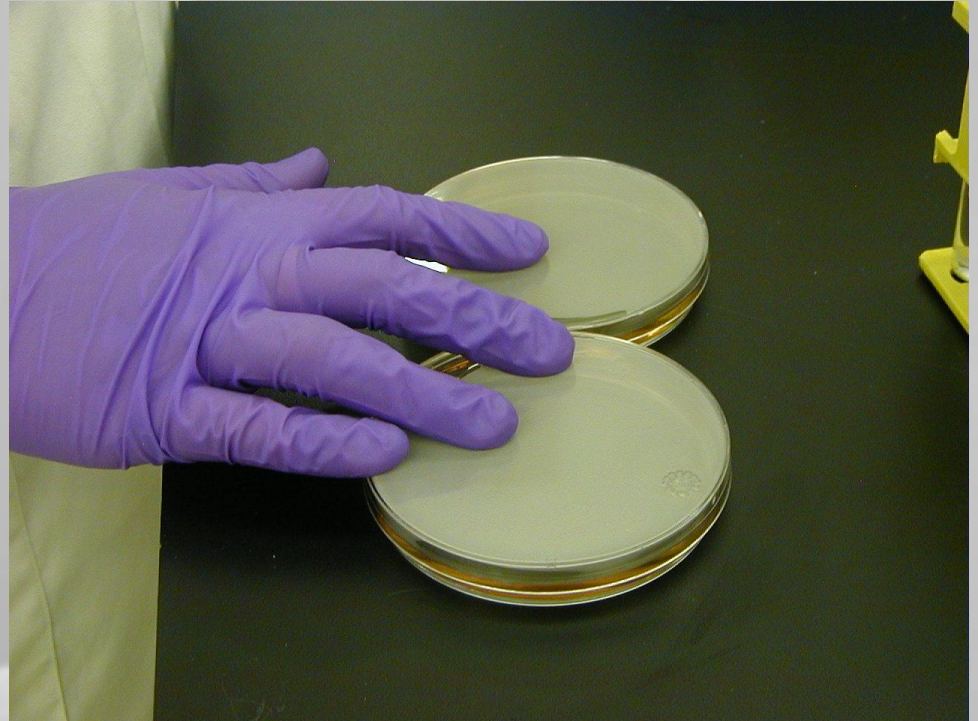
Step 12: Pour approximately 20 ml of melted soybean casein digest agar, cooled to 45° to 50° C, into the petri plate.



Step 13:

Swirl the agar filled plates to assure adequate mixing. Allow the agar to solidify.

Do not use agar that has been melted and held longer than eight (8) hours.



Step 14: Invert and incubate plates at the appropriate temperature for forty-eight (48) hours.

Step 15: After forty-eight (48) hours of incubation, remove plates and count colonies. Counts should be between 30 – 300 CFU per plate.



A 2.0×10^6 population will yield 80 CFUs on a 10^{-5} plate.

Species	Incubation Temp.
<i>G. stearothermophilus</i>	55 - 60°C
<i>B. subtilis</i> “5230”	30 - 35°C

Step 16: After counting the CFU's per plate, calculate the overall average recovered CFU's from all of the plates. Record the data in the following table.

Carrier/Tube Number	Replicate Plate #1	Replicate Plate #2	Average of Plates 1 and 2
1			
2			
3			
4			
Overall average CFU per plate			

Step 17: Calculate the average population recovered from the carrier lot samples by multiplying the calculated overall average CFU per plate by the inverse of the dilution factor.

Equation: overall average plate count CFU x $\frac{1}{\text{dilution factor}}$

Example 1: If the expected population from the carrier lot is 10^5 , then the dilution factor should be 10^{-4} , as shown previously. If the overall average plate count is 80 CFU, then the *calculated average population* of the samples is as follows:

$$80 \text{ CFU's} \times \frac{1}{10^{-4}} = 80 \times 10^4 = 800,000 = 8 \times 10^5 \text{ CFU/4 ampoules} = 2.0 \times 10^5/\text{ampoule}$$

Example 2: If the expected population from the carrier lot is 10^6 , then the dilution factor should be 10^{-5} , as shown previously. If the overall average plate count is 80 CFU, then the *calculated average population* of the samples is as follows:

$$80 \text{ CFU's} \times \frac{1}{10^{-5}} = 80 \times 10^5 = 8,000,000 = 8 \times 10^6 \text{ CFU/4 ampoules} = 2.0 \times 10^6/\text{ampoule}$$

Step 18: To confirm the population stated on the certificate, the percent recovery of the certified population must be within -50% , $+300\%$ in accordance with USP and ISO 11138. Take the average population determined by the assay and divide by the population value stated on the certificate.

$$\text{Percent recovery} = \frac{\textit{calculated average population of samples}}{\textit{stated population on certificate}} \times 100$$

If stated certificate population is 1.0×10^5 , then a recovered population within the range of $5.0 \times 10^4 - 3.0 \times 10^5$ is acceptable.

If stated certificate population is 1.0×10^6 , then a recovered population within the range of $5.0 \times 10^5 - 3.0 \times 10^6$ is acceptable.

NOTE: If recovery is not confirmed within -50% , $+300\%$ of the certificate value, examine possible calculation and/or procedural errors.

If the variation between plates is greater than 30 CFU's, re-examine technique used.