

POPULATION ASSAY: PROSPORE AMPOULE

LOT#: _____ ORGANISM: *G. stearothermophilus* Other _____

Fill Volume (circle one): 1ml 4 ml 1.9 ml SPS _____ ml TSA Lot # _____

LABELED POP/POPULATION LEVEL: _____

PROCEDURE:

- 1.0 Vortex the ampoule for 1 minute in an Upside Down Position, and then for 1 minute in Normal Vertical Position being careful to wash out any spores that may be adhering to the glass in the upper tip of the ampoule. This step is necessary to achieve an accurate population count. Then aseptically transfer a 1ml aliquot to a sterile, screw-capped, 10 ml test tube containing 9 ml of sterile, processed water.
- 2.0 Heat shock in a water bath (10 minutes at 80°-85°C for mesophiles and 15 minutes at 95° - 100°C for *G. stearothermophilus* and other thermophiles). Immediately cool in a water bath of 0° - 4°C.

Start Time/Temperature: _____ / _____ °C **End Time:** _____

Initial and Date: _____ / _____

- 3.0 Vortex the tube for 15-20 seconds.
- 4.0 Perform serial dilutions by pipetting out 1.0 ml of the aliquot into another sterile, screw-capped 10 ml test tube containing 9.0 ml of sterile, processed water. Repeat from step 3 until desired dilution factor is reached.
- 5.0 From the next-to-the-last dilution, pipette out 1.0 ml into each of three Petri plates. Repeat for the final dilution.
- 6.0 Within 20 minutes, add approximately 20 ml TSA, pre-sterilized and cooled to 47° ± 2°C. Swirl to distribute spores evenly in agar and allow to solidify.

TSA Temperature: _____ °C **Initial and Date:** _____ / _____

- 7.0 Invert and incubate the plates (30°-35°C for mesophiles, 55°-60°C for *G. stearothermophilus* and other thermophiles).

Incubation Start Time/Initial & Date: _____ / _____ **Incubator #:** _____

- 8.0 Examine all plates at 24 (±1) hours. Record on the back the number of colony forming units (CFU's) per plate. Record the average on the following page.
- 9.0 Calculate the average number of CFUs/ampoule from the above data using the formula on the following page.

Performed By: _____ **Date:** _____

POPULATION ASSAY: PROSPORE AMPOULE

Total @ 24 hrs / number of plates counted x DF x AVF = CFU/ampoule
 DF= Dilution factor (absolute value of the reciprocal of the dilution)
 AV= Average number of colonies per ampoule
 AFV=Ampoule fill volume

Incubation End Time/Initial & Date: _____/_____

CFU COUNTS AT 24 HOURS

dilutions _____

24hrs

Plates 1. _____ 2. _____ 3. _____ Total @ 24hours: _____

Total @ 24 hrs _____ / 3 x _____(DF) x _____(AFV) = _____(AV)CFU/ampoule

CFU COUNTS AT 24 HOURS

dilutions _____

24hrs

Plates 1. _____ 2. _____ 3. _____ Total @ 24 hours: _____

Total @ 24 hrs _____ / 3 x _____(DF) x _____(AFV)= _____(AV)CFU/ampoule

of Dilutions = Dilution Factor

- 1 = 10
- 2 = 100
- 3 = 1000
- 4 = 10000
- 5 = 100000
- 6 = 1000000

Sum of the AV of both dilution / 2 =CFU/ ml

_____ / 2 =

_____ x10 _____ CFU/ampoule

Read By: _____ Date: _____