

POPULATION ASSAY: SPORE SUSPENSION

LOT #: _____ LABELED POP/POP LEVEL _____

ORGANISM (circle one): _____ TSA Lot # _____

B. atrophaeus *G. stearothermophilus* *B. pumilus* Other _____

PROCEDURE:

- 1.0 Vortex the suspension for 2 minutes, then aseptically transfer a 1.0 ml aliquot into a sterile, screw-capped 10 ml test tube containing 9.0 ml of sterile, processed water.
- 2.0 Heat shock tubes in a water bath (10 minutes at 80°-85°C for *B. atrophaeus*/*B. pumilus* and other mesophiles, 15 minutes at 95°-100°C for *G. stearothermophilus*). Immediately cool tubes in a water bath of 0° - 4°C.

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

Initial and Date: _____ / _____

- 3.0 Vortex the tubes 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 *B. atrophaeus*/*B. pumilus* and other mesophiles, 55°-60°C for *G. stearothermophilus*).

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 CFU's per ml from the above data by the formulas on the following page:

Performed By: _____ Date: _____

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Total @ 24 hrs / number of plates counted x DF = CFU/ml
 DF= Dilution factor (absolute value of the reciprocal of the dilution)
 AV= Average number of colonies per ml

Incubation End Time/Initial & Date: _____ / _____

CFU COUNTS AT 24 HOURS

dilutions _____

24hrs

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

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

CFU COUNTS AT 24 HOURS

dilutions _____

24hrs

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

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

of Dilutions = Dilution Factor

- 1 = 10
- 2 = 100
- 3 = 1000
- 4 = 10000
- 5 = 100000
- 6 = 1000000
- 7 = 10000000
- 8 = 100000000
- 9 = 1000000000

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

_____ / 2 =

_____ x10^{_____} CFU/ml

Read By: _____ Date: _____