

# POPULATION ASSAY: SPORE SUSPENSION

LOT #: \_\_\_\_\_ LABELED POP/POP LEVEL \_\_\_\_\_

ORGANISM (circle one): *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*, *B. smithii* and other thermophiles). 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. Also pour 1 Media Negative Control plate.

TSA Temperature: \_\_\_\_\_ °C TSA Lot #: \_\_\_\_\_

Initial and Date: \_\_\_\_\_ / \_\_\_\_\_

- 7.0 Invert and incubate the plates (30°-35°C for *B. atrophaeus*/*B. pumilus* and other mesophiles, 48°-52°C for *B. smithii*, 55°-60°C for *G. stearothermophilus*). Incubate Media Negative Control at same temperature as assay.

Incubation Start Time: \_\_\_\_\_ Incubator #: \_\_\_\_\_

Initial and Date: \_\_\_\_\_ / \_\_\_\_\_

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8.0 Examine all plates at 24 ( ± 1) hours and record the number of colony forming units (CFU's) per plate. Calculate the average number of CFU's per carrier by using the formulas below.

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 @ 24hours: \_\_\_\_\_

Total @ 24 hrs \_\_\_\_\_ / 3 x \_\_\_\_\_ (DF) = \_\_\_\_\_ (AV)CFU/ml  
 \_\_\_\_\_ (4 decimals)

### CFU COUNTS AT 24 HOURS

# dilutions \_\_\_\_\_

**24hrs**

Plates 1. \_\_\_\_\_ 2. \_\_\_\_\_ 3. \_\_\_\_\_ Total @ 24hours: \_\_\_\_\_

Total @ 24 hrs \_\_\_\_\_ / 3 x \_\_\_\_\_ (DF) = \_\_\_\_\_ (AV)CFU/ml  
 \_\_\_\_\_ (4 decimals)

# of Dilutions = Dilution Factor

1 = 10

2 = 100

3 = 1000

4 = 10000

5 = 100000

6 = 1000000

7 = 10000000

8 = 100000000

9 = 1000000000

TFTC=Too few to count <10CFUs

TNTC=Too numerous to count >300CFUs

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

Media Negative Control: \_\_\_\_\_

\_\_\_\_\_ / 2 =

\_\_\_\_\_ x10 \_\_\_\_\_ CFU/ml (4 decimals)

Read By: \_\_\_\_\_ Date: \_\_\_\_\_

Reviewed By: \_\_\_\_\_ Date: \_\_\_\_\_