

POPULATION ASSAY: PROAMP

LOT#: _____ POPULATION LEVEL: _____

Fill Volume: _____ ml

ORGANISM (circle one): *G. stearothermophilus* *B. subtilis* Other _____

PROCEDURE:

- 1.0 Aseptically wipe down 5 ProAmps with 70% Isopropyl alcohol and place in sterile 50mL conical tube. Using a sterile pestle or other suitable device, aseptically crush the ampoules. Aseptically withdraw 1.0mL of the pooled solution and place into a sterile screw-capped 10mL test tube containing 9.0mL 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 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 At the dilution expected to yield 10-300 CFU, pipette out 1.0mL into each of three Petri plates. Repeat for the final dilutions.
- 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 Lot # _____ TSA Temperature: _____ °C

Initial and Date: _____ / _____

- 7.0 Invert and incubate the plates (30°-35°C for mesophiles, 55°-60°C for thermophiles). Incubate Media Negative Control plate 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 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 _____

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

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

CFU COUNTS AT 24 HOURS

dilutions _____

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

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

of Dilutions = Dilution Factor

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

TFTC = Too few to count <10CFUs
 TNTC = Too numerous to count >300CFUs

Media Negative Control: _____

Sum of the AV of both dilutions / 2 =CFU/ ampoule

_____ / 2 =

_____ x10 _____ CFU/ampoule (4 decimals)

Read By: _____ Date: _____

Reviewed By: _____ Date: _____