POPULATION ASSAY:
PROSPORE AMPOULE

LOT#: _____ LABELED POP/POP LEVEL: _____

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

Fill Volume (circle one): 1ml 4 ml 1.9 ml SPS Other _____ ml

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 80º-85ºC for mesophiles and 15 minutes at 95º - 100ºC for *G. stearothermophilus* and other thermophiles). Immediately cool in a water bath of 0º - 4ºC.

Start Time/Temperature:_____/_____ºC 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 47º $\pm$ 2ºC. Swirl to distribute spores evenly in agar and allow to solidify. Also pour 1 Media Negative Control Plate.

TSA Temperature:_____ºC TSA Lot #:_____

Initial and Date:_____/_____

7.0 Invert and incubate the plates (30º-35ºC for mesophiles, 55º-60ºC for *G. stearothermophilus* and other thermophiles). 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 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
(4 decimals)

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
(4 decimals)

# of Dilutions = Dilution Factor
1 = 10
2 = 100
3 = 1000
4 = 10000
5 = 100000
6 = 1000000

Media Negative Control:_____

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

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

_____ / 2 =

_____x10 ___ CFU/ampoule (4 decimals)

Read By:_____Date:_____

Reviewed By:_____Date:_____