POPULATION ASSAY:
SPORE STRIPS/DISC/THREAD/SUTURE

Lot # _____ POP LEVEL _____

CARRIER (circle one): Spore Strip Disc Thread Suture Other _____

ORGANISM(S): *B. atrophaeus* *G. stearothermophilus* Other _____

PROCEDURE:

1.0 Aseptically transfer 10 spore strips/discs/threads/sutures into sterile 250 ml blender cup containing 100 ml chilled processed water

2.0 Blend 3-5 minutes to a homogeneous pulp of component fibers. [For threads and sutures stop the blender at one minute intervals and check the blades to ensure that the threads or sutures are not stuck to them. If the threads or sutures are stuck to the blade, shake the cup to dislodge and continue with blending.]

3.0 Aseptically transfer a 10 ml aliquot from the blender cup into a sterile, screw-capped 10 ml test tube. Label each tube with lot #, temperature and length of exposure.

4.0 Heat shock tubes in a water bath (10 minutes at 80º - 85ºC for *B. atrophaeus* and other mesophiles, 15 minutes at 95º - 100ºC for *G. stearothermophilus*.) Immediately cool tubes in a water bath of 0º - 4ºC.

Start Time/Temperature:_____/_____ºC End Time:_____

Initial and Date:_____/_____

5.0 Vortex the tubes for 15-20 seconds.

6.0 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 5 until desired dilution factor is reached.

7.0 At the dilution factors expected to yield 10-300 CFU, pipette out 1.0 ml into each of three petri plates. Repeat for final dilutions.

8.0 Within 20 minutes, add to each plate approximately 20 ml of 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:_____/_____

9.0 Invert and incubate the plates (30º - 35ºC for *B. atrophaeus* and other mesophiles, 55º - 60ºC for G. *stearothermophilus*). Incubate Media Negative control at same temperature as assay.

Incubation Start Time:_____Incubator #_____

Initial and Date: _____/_____

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10.0 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/spore carrier
DF= Dilution factor (absolute value of the reciprocal of the dilution)
AV= Average number of colonies per spore carrier

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/Spore carrier
(4 decimals)

CFU COUNTS AT 24 HOURS

dilutions _____

24hrs

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

Total @ 24 hrs_____ / 3 x _____(DF) =_____(AV)CFU/Spore carrier
(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/ Spore carrier

_____ / 2 =

_____x10 ___ CFU/Spore Carrier (4 decimals)

Read By:_____Date:_____

Reviewed By:_____Date:_____