POPULATION ASSAY:
SPORE STRIPS/DISC/THREAD/METAL

Lot # _____POP LEVEL _____TSA Lot # _____

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

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

PROCEDURE:

1.0 Aseptically transfer 10 spore strips/discs/threads into sterile 250 ml blender cup containing 100 ml chilled processed water. [If processing metal product, aseptically transfer 1 carrier into a water blank containing 9.9 ml sterile, processed water with 0.1 ml of Tween 80 and 1ml of 3mm sterile glass beads. Vortex for 2 minutes. Insert 10 ml tube into sonicator (38.5 – 40.5 KHz, full wave industrial stack transducer) for 10 minutes. Vortex again for 2 minutes and skip to step 4.0)]

2.0 Blend 3-5 minutes to a homogeneous pulp of component fibers.

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*, 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, screwcapped 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 ± 2ºC. Swirl to distribute spores evenly in agar and allow to solidify.

TSA Temperature:_____ºC Initial and Date:_____/_____

9.0 Invert and incubate the plates (30º - 35ºC for *B. atrophaeus/B. pumilus* and other mesophiles, 55º - 60ºC for
G. *stearothermophilus*).

Incubation Start Time/Initial & Date:_____/_____Incubator #_____

10.0 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.

11.0 Calculate the average number of CFU's per carrier from the above data using the formulas on the following page:

Performed By:_____ Date:_____

POPULATION ASSAY:
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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

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CFU COUNTS AT 24 HOURS

dilutions _____

24hrs

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

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

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

Sum of the AV of both dilution / 2 =CFU/ Spore carrier

_____ / 2 =

_____x10__ CFU/Spore Carrier

Read By:_____Date:_____