POPULATION ASSAY:
METAL DISCS/METAL STRIPS/WIRES

LOT # _____ LABELED POP _____

CARRIER (circle one): Strip Disc Wire Other: _____

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

PROCEDURE:

1.0 Aseptically transfer 1 disc, strip or wire into a water blank containing 10 ml sterile, processed water. Vortex for not less than (NLT) 2 minutes.

2.0 Allow tube to soak NLT 15 minute in refrigerator, longer soaking is optional.

3.0 Remove tube from refrigerator and vortex NLT 15 seconds.

4.0 Insert into sonicator (42 kHz ±6%, full wave industrial stack transducer) for 7 minutes, rotate tube to another location in rack and sonicate an additional 8 minutes (NLT 15 minutes total).

Note: Tubes must not be placed next to each other in rack. 5 tubes maximum.

5.0 Heat shock tubes in a water bath (10 minutes at 80º - 85ºC for *B. atrophaeus*, 15 minutes at 95º-100ºC for *G. stearothermophilus*.)

Note: Ensure water level in bath is above the water level of the tube. 5 tube maximum.

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

Initial and Date: _____/_____

6.0 Remove tubes and cool in an ice water bath for 5-10 minutes. 60 minutes maximum.

7.0 Vortex the tube NLT 15 seconds and 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 this step until desired dilution factor is reached.

8.0 From the next-to-last dilution, pipette out 1.0 ml into each of three petri plates. Repeat for final dilution.

9.0 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. Also pour one Media Negative control plate.

TSA Temperature:_____ºC TSA Lot #:_____

Initial and Date: _____/_____

10.0 Invert plates and place back in to petri plate bag. Fold bag over once and tape closed.

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

Incubation Start Time: _____ Incubator #:_____

Initial and Date:_____/_____

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12.0 Examine all plates at 24 (±1) hours. Count the number of colony forming units (CFU's) per plate. Record the totals and averages below.

13.0 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:_____

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

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

_____ / 2 =

_____x $10^{\_\_}$ CFU/Spore Carrier (4 decimals)

Read By:_____Date:_____

Reviewed By:_____ Date:_____