STABILITY POPULATION ASSAY: METAL DISCS/METAL STRIPS/WIRES

LOT # _____ LABELED POP _____

CARRIER (circle one): Strip Disc Wire TSA Lot #_____

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

PROCEDURE:

1.0 Aseptically transfer 1 disc, strip or wire 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. Place in refrigerator overnight.

2. Remove 10 ml tube from refrigerator and vortex for 2 minutes. Insert into sonicator (38.5 – 40.5 KHz, full wave industrial stack transducer) for 10 minutes.

3.0 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:_____/_____

4.0 Vortex the tube for 1 minute. **IMPORTANT:** Make sure that metal carrier does not get stuck in the tube during vortexing. The carrier **must** flow freely with the glass bead. **Prior to performing serial dilutions, visually check to make sure that the spore deposit has been completely removed from the carrier.**

5.0 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 3 until desired dilution factor is reached.

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

7.0 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.

TSA Temperature:_____ºC Initial and Date:_____/_____

8.0 Invert and incubate the plates (30º - 35ºC for *B. atrophaeus;* 55º - 60ºC for *G. stearothermophilus*).

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

9.0 Examine all plates at 24 ($\pm$1) hours. Record on the back the number of colony forming units (CFU's) per plate. Record the average on the following page.

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

Performed By:_____Date:_____

STABILITY POPULATION ASSAY:
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Total @ 48 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 @ 48hours: _____

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 =

_____x ~~10~~ CFU/Spore Carrier

Read By:_____Date:_____