

Population Assay of Biological Indicator Products

1.0 Purpose

The purpose of this work instruction is to provide details on how to perform population assays on Mesa Labs Omaha Manufacturing Facility's biological indicators (BIs) containing maceratable carriers.

2.0 Scope

This SOP applies to all Mesa Labs personnel who perform population assays.

3.0 Acronyms

- BI – Biological Indicator
- TSA – Tryptic Soy Agar
- CFU – Colony Forming Units
- RO/DI – Reverse Osmosis/Deionized water
- NLT – Not less than

4.0 Responsibility & Authority

- 4.1 The Area Supervisor/Manager is responsible for ensuring this work instruction is followed and that laboratory personnel are properly trained in the related procedures.
- 4.2 Laboratory personnel are responsible for following this work instruction when performing population assays on paper carrier and spore thread BIs.

5.0 Procedure

5.1 General

- 5.1.1 Population assays for product release, will be performed by the number of qualified technicians as specified on the associated production record.
- 5.1.2 Population assays for contract testing will be performed by qualified personnel as directed in the appropriate protocol or production record.
- 5.1.3 The average of the assays is calculated on LF-1601 Labeled Population Worksheet.
- 5.1.4 Any volume between 0.5mL and 2mL may be plated to achieve 10-300 CFUs per plate.
- 5.1.5 Lower population products may require a different volume to achieve 10-300 CFUs per plate.
- Note: for Contract Testing population assays, any volume between 0.5ml and 5ml may be plated to achieve 30-300 CFUs per plate.**
- 5.1.6 Plates are made by pouring approximately 20mL of TSA, pre-sterilized and cooled to 47° +/- 2°C. Swirl to distribute spores evenly in agar and allow to solidify.

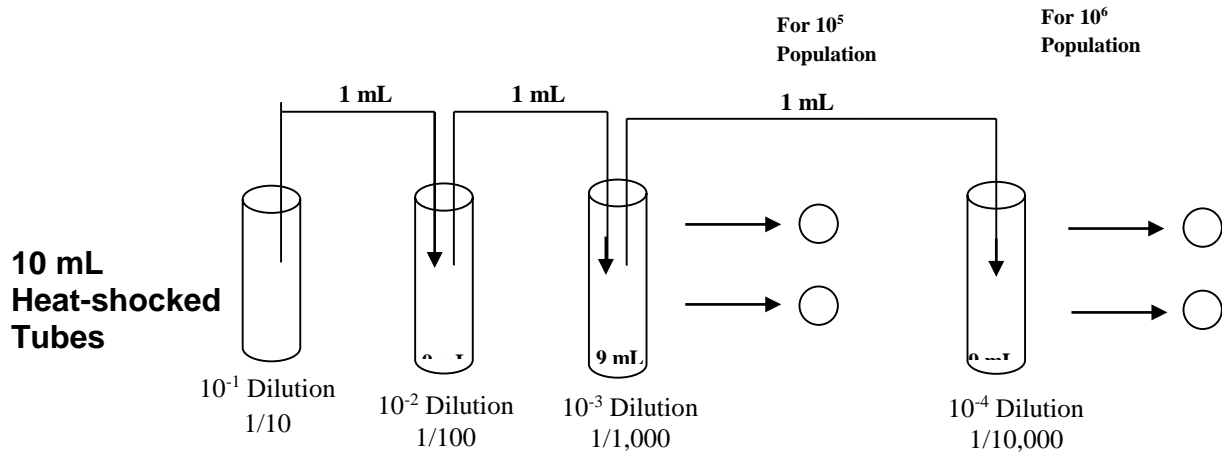
5.1.6.1 A Media Negative Control plate should also be prepared upon completion of the assay. This is done by pouring approximately 20mL of TSA into an empty petri plate and incubating it with the plates from the assay.

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- 5.1.7 When plates have solidified invert and incubate at the appropriate temperature for 24 hours +/- 1 hour for products for release and for 48 hours +/- 2 hours for contract testing products.
- Thermophiles shall be incubated at 55-60°C, mesophiles at 30-35°C and *B. smithii* at 48-52°C.
- 5.2 Glass Bead Method (for inoculated paper, quartz, borosilicate and cotton thread carriers)
- 5.2.1 Randomly select four inoculated carriers from the lot to be assayed.
- 5.2.2 Place each individual carrier in to each of four sterile, screw cap, flat-bottom tube with four 6mm glass beads and 5mL of sterile RO/DI water.
- 5.2.3 Vortex for NLT four minutes until the BI is macerated to pulp. It may take longer than four minutes to achieve desired results.
- 5.2.4 Add 5mL of sterile RO/DI water and vortex NLT 10 seconds.
- 5.2.5 Heat shock the tubes (10 minutes at 80° – 85°C for *B. atrophaeus* and other mesophiles, 15 minutes at 95° – 100°C for *G. stearothermophilus* and other thermophiles) starting the timing when the heat shock check thermometer reaches the desired temperature.
- 5.2.6 Immediately cool the tubes in a water bath of 0° – 4°C.
- 5.2.7 Dilution Series (10⁵ and 10⁶ population):
- Note: this section outlines the dilution scheme for 10⁵ and 10⁶ biological indicators. For product with populations that fall outside of that range the number of dilutions will need to be adjusted up or down accordingly.**
- 5.2.7.1 A dilution series will be made from each tube.
- 5.2.7.2 Vortex each heat-shocked tube NLT 10 seconds.
- 5.2.7.3 From each tube, transfer a 1mL aliquot to a dilution tube containing 9mL of sterile RO/DI water.
- 5.2.7.4 Vortex the dilution tube NLT 10 seconds.
- 5.2.7.5 Transfer 1mL to a second dilution tube containing 9mL of sterile RO/DI water.
- 5.2.7.6 Repeat 5.2.7.4-5.2.7.5 one more time for a 10⁶ population.
- 5.2.7.7 Vortex this tube for NLT 10 seconds.
- 5.2.7.8 Pipette from this dilution tube into two petri plates.
- 5.2.7.9 Refer to Figure 1 for a schematic of this dilution series.
- 5.2.8 After incubation, count colonies.
- 5.2.9 Average counts and then multiply by the inverse of the dilution (i.e. the dilution factor) to calculate population per original unit. If an amount was plated other than 1mL be sure to account for this in the calculations (i.e. if 0.5mL was plated then the totals must be multiplied by 2, if 2mL was plated then the totals must be divided by 2, etc.). Record all on LF-3612.

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Figure 1



6.0 Records

6.1 All population assays for product release shall reside in the corresponding batch records.

6.2 All population assays for contract testing shall reside in the appropriate contract binder.

Regulatory References	
QAL-0701	Standards Log
Referenced Documents	
LF-3612	Population Data Sheet
LF-1601	Labeled Population Worksheet