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1.0 Purpose

This Standard Operating Procedure (SOP) describes microbial sterile membrane filtration according to current USP standards utilizing the Millipore Milliflex Test System(s) to determine the presence/absence of coliforms in a test sample utilizing m-Endo LES agar that is recommended for the cultivation of coliform bacteria.

2.0 Scope

This SOP applies to Process Analytics (PA) personnel who will be performing this procedure.

3.0 Authority and Responsibility

- 3.1 The Director, PA has the authority to define this procedure.
- 3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 PA personnel are responsible for the performance of this procedure.
- 3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.
- 3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials

- 4.1 m-Endo LES Agar (LES) media cassette, BDP PN 21886.

5.0 Procedure

- 5.1 Process 100 mL of test sample according to **SOP 22133, *Bioburden Assay by the Membrane Filtration Method.***
- 5.2 Apply an m-Endo LES Agar media cassette to the processed funnel unit.
- 5.3 Incubate in the inverted position at 30 – 35°C for 24 – 72 hours.



- 5.4 Incubate a negative control agar plate with each day's sample set. Plates will be tested for growth promotion as per **SOP 22712, *Media Qualification Testing***.
- 5.5 After the required incubation period, count the number of coliform colonies and record the data on Form 22159-01. Coliform colonies appear deep red to red-black with a distinct golden-green metallic sheen.

6.0 Identification of Isolates (Optional)

- 6.1 Colonies are speciated if requested.
- 6.2 For speciation of isolates, streak each morphologically unique isolate onto a TSA plate and incubate at the original isolation incubation temperature overnight. The isolate may be sent to a vendor supplying identification services or identified in-house.

7.0 Documentation

- 7.1 Record all results on Form 22159-01. Only report the number of coliform colonies seen.
- 7.2 The results are reviewed by PA and BQA.
- 7.3 The original data is archived with the QC Test Request Form in BQA as per **SOP 21402, *Archived Documents***.
- 7.4 Record use and maintenance activities in the equipment logbook.

8.0 References and Related Documents

SOP 21402 *Archived Documents*

SOP 22133 *Bioburden Assay by the Membrane Filtration Method*

SOP 22712 *Media Qualification Testing*

Form 22159-01 m-Endo LES Agar Coliform Assay

Milliflex Test System Operation, Sanitation, and Maintenance Instruction.

Millipore Pre-filled Milliflex Cassette with m-Endo LES Agar Product Insert.

9.0 Change Summary

