Biopharmaceutical Development Program

Standard Operating Procedure

Title: Milliflex-Sensor II Coliform Assay

SOP Number: 22159 **Revision Number: 01**

Supersedes: Revision 00 **Effective Date: SEP 15 2011**

Originator/Date: [redacted]

Approval/Date:

Approval/Date:

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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-100 Test System 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).

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

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, calibration, and maintenance activities in the Milliflex-Sensor II equipment logbook.

8.0 References and Related Documents

8.1 **SOP 21402** *Archived Documents*

8.2 **SOP 22133** *Bioburden Assay by the Membrane Filtration Method*

8.3 **SOP 22712** *Media Qualification Testing*

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8.4 Current USP.

8.5 Milliflex-100 Test System Operation, Sanitation, and Maintenance Instruction.

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

9.0 Attachments

9.1 **Attachment 1** Form 22159-01, Milliflex-Sensor II Coliform Assay

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Attachment 1

NCI-Frederick
Form No.: 22159-01
SOP No.: 22159
Revision 01:

Milliflex-Sensor II Coliform Assay

m-Endo LES Agar Cassette Lot Number/Expiration Date: _____

PBS/Sterile Water Lot Number/Expiration Date: _____

30-35°C Incubator Number: _____ Calibration Due Date: _____

Incubation Start Temp: _____ Incubation Stop Temp: _____

Incubation Start Date/Time: _____ Incubation Stop Date/Time: _____

Sample Name	QC Test Request #	Amount Filtered	# of Coliform Colonies
Negative Control		100 mL	

Comments:

_____

_____

_____

_____

Assay Performed By/Date: _____

Assay Read By/Date: _____

PA Review By/Date: _____

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