Biopharmaceutical Development Program

Standard Operating Procedure

Title: Microbial Content

SOP Number: 22713 **Revision Number: 02**

Supersedes: Revision 01 **Effective Date: DEC 10 2010**

Originator/Date: [redacted]

Approval/Date:

Approval/Date:

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1.0 Purpose

This procedure describes how to determine the number of organisms in a sample.

2.0 Scope

This procedure is to be conducted by trained Process Analytics (PA) staff in PAlaboratories of the Biopharmaceutical Development Program (BDP).

3.0 Authority and Responsibility

3.1 The Director, Process Analytics (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.

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

3.5 Biopharmaceutical Quality Assurance is responsible for quality oversight of this procedure.

4.0 Materials

Note: An approved equivalent to the following may be used.

4.1 Tryptic Soy Agar (TSA) plates (BDP PN 10006).

4.2 Sterile Phosphate Buffered Saline (BDP PN 10139).

4.3 Plastic disposable spreaders or loops (BDP PN 20505).

4.4 Sterile dilution tubes (BDP PN 20317).

4.5 Pipettor with 1 mL (BDP PN 20101) and 10 mL (BDP PN 20100) sterile pipettes.

4.6 Parafilm (BDP PN 20464, 20465) or Ziploc™ bags (BDP PN 20339, 20340).

4.7 Laminar Flow Biosafety Hood.

4.8 $35 \pm 3°C$ Incubator.

4.9 Vortex (optional).

5.0 Procedure

Perform all manipulations in a laminar flow biosafety hood. Observe all principles pertaining to aseptic technique when performing this procedure. Samples are identified by their QC ID number and their individual numbers.

5.1 Warm all plates and samples to room temperature before performing any manipulations.

5.2 Label the bottoms of the TSA plates with appropriate designations as to the date, the dilution which is being put onto the particular plate, and the identification of the sample.

5.3 If necessary, and there is $\geq$ 2.3 mL of sample available, make serial 10-fold dilutions in sterile phosphate buffered saline (diluent) to a final dilution of 10^{-6}. **Make certain to change pipette tips between each dilution**. Vortex thoroughly.

Example:

10-1dilution: 0.3 mL of sample plus 2.7 mL of saline;

10-2dilution: 0.3 mL of the 10^{-1} dilution plus 2.7 mL of saline; and so on for six serial dilutions.

5.4 After the dilutions have been made, pipet 1.0 mL of each dilution onto each of two individual TSA plates, starting with the greatest dilution (10^{-6}) and working toward the lowest dilution (10^{-1}). Manipulate each plate so that the liquid is uniformly distributed over the entire area of the plate. Allow the plates to dry.

5.5 Pipet 1.0 mL of the undiluted sample onto each of two individual TSA plates. This is designated as the 10° dilution. Manipulate each plate so that the liquid is uniformly distributed over the entire area of the plate. Allow the plates to dry.

5.6 As a negative control, prepare two plates by inoculating with the sterile media or buffer used to formulate the sample(s), or with diluent if the first option is not available. Allow the plates to dry.

5.7 Parafilm each plate or put the plates into a Ziploc™ bag. Invert the plates and incubate 18 hours – 5 days at 35°C ± 3°C.

5.8 Count the individual colonies on each plate after 18 hours – 5 days of incubation. Make any notations if there appears to be more than one colony morphology on an individual plate.

5.9 If dilutions are used, select plates with a colony count between 30 and 300. Use selected plates with the least number of colonies to determine the titer. If a colony count of at least 30 is not achieved, select the plates with the highest number of colonies to determine the titer.

(#Colonies plate 1) + (#Colonies plate 2) ÷ by (2) x (Dilution of the plate) = $\frac{\text{CFU}}{\text{mL}}$

Example:

Dilution	Plate 1	Plate 2
10^{0}	Too numerous to count (TNTC)	TNTC
10^{-1}	TNTC	TNTC
10^{-2}	291	289
10^{-3}	31	37
10^{-4}	3	8
10^{-5}	0	1
10^{-6}	0	0

The 10^{-3} plates had the smallest number of countable colonies within the acceptable range. The titer in this example would be 3.4 10^{4} CFU/mL.

5.10 Save the plates at 2 - 8°C for at least 30 days in case an identification is requested for the different colony morphologies on the plates.

6.0 Documentation

6.1 Record the data on Form 22713-01. Note any observations about the colony morphologies on the plates.

6.2 The document is reviewed by PA. Form 22713-01 is attached to the QC Test Request form and submitted for final review and approval.

6.3 Raw data is archived with the QC Test Request Form in BQA as per **SOP 21407, *Records Retention***.

7.0 References and Related Documents

7.1 **SOP 21407** *Records Retention*

8.0 Attachments

8.1 **Attachment 1** Form 22713-01, Microbial Content

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Attachment 1

NCI-Frederick
Form No.: 22713-01
SOP No.: 22713
Revision 02:

Microbial Content

QC ID#:_____ Date:_____

Project Name/Number:_____

Sample Name/Lot #:_____ Amount of Dilution Plated:_____

Media/Lot#/Expiration Date:_____

Diluent/Lot#/Expiration Date:_____

Incubator MEF #: _____Calibration Due Date: _____

Incubation Start Date/Time: _____ Incubation End Date Time:_____

Sample Dilutions	Colonies Counted Plate 1	Colonies Counted Plate 2
Control		
10^{0}		
10^{-1}		
10^{-2}		
10^{-3}		
10^{-4}		
10^{-5}		
10^{-6}		

Incubation Time: _____

Colony Morphology: _____Uniform _____Non-uniform

CFU/mL:_____

Comments:_____

_____

_____

_____

Performed By/Date:_____

PA Review By/Date:_____

BQA Review By/Date:_____

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