SOP Title: **Microbial Content**
SOP Number: **22713**
Revision: **03**

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1. PURPOSE

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

2. SCOPE

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

3. RESPONSIBILITIES

3.1 Director / Process Analytics (PA)

- Defines procedure

3.2 Process Analytics (PA) Personnel

- Provides training
- Performs procedure
- Records and reviews data

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
10006	Tryptic Soy Agar (TSA) plates	☒ YES ☐ NO
10139	Sterile Phosphate Buffered Saline	☒ YES ☐ NO
20505	Plastic disposable spreaders or loops	☒ YES ☐ NO
20317	Sterile dilution tubes	☒ YES ☐ NO
20101	Pipettor with 1 mL	☒ YES ☐ NO
20100	10 mL sterile pipettes	☒ YES ☐ NO
20464, 20465	Parafilm	☒ YES ☐ NO
20339, 20340	Ziploc™ bags	☒ YES ☐ NO

5. EQUIPMENT

- Laminar Flow Biosafety Hood.
- 35 ± 3°C Incubator
- Vortex (optional)

6. PROCEDURE OR USE

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.

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

6.2 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.

6.3 If necessary, and there is ≥ 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.

Additional steps Example:

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

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

6.4 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.

6.5 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.

6.6 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.

6.7 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.

6.8 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.

6.9 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.

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

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.

6.10 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.

7. DOCUMENTATION AND RECORDS

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

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

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

8. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
21407	Records Retention
22713-01	Microbial Content