ION LABS	Standard Operating Procedure **Determining Phage Titer**	**SOP Number** **D-1015**	**Revision** **0**
		Effective Date 01/03/23	**Page** **Page 1 of 9**
Written by/ Date 12/12/22	**Reviewed by/ Date** 12/20/22	**Approved by/ Date** 12-20-22	
Title: Senior Microbiologist	**Title: Quality Control Director**	**Title: VP of Quality & Regulatory Affairs**	

1.0 Purpose

The purpose of this procedure is to define the process for determining the plaque-forming unites per gram (PFU/g) of phage in a given sample. When an individual bacterial virus grows in a bacterial host suspended in a top agar "lawn", its progeny infects and lyses the surrounding host cells. This causes the appearance of a "hole" or plaque in the bacterial lawn, where each plaque represent a single virus.

2.0 Scope

This procedure describes the determination of PFU/g in raw material and final product samples containing phage.

3.0 Responsibility

3.1 It is the responsibility of QC Laboratory Management and/or designee to implement and maintain this procedure and to ensure that the procedure is followed.

3.2 It is the responsibility of QC Laboratory Management and/or designee to review and approve all qualification and test documentation.

3.3 It is the responsibility of QC Laboratory Microbiologists to train on this procedure before any testing occurs.

4.0 Definitions

4.1 **PFU/g** – plaque forming units per gram

4.2 **QC** – Quality Control

5.0 References

5.1 PRTCL-22-0055, Protocol, Transfer of Determining Phage Titer to the Ion Labs Microbiology Laboratory

5.2 RPT-22-0026, Report, Transfer of Determining Phage Titer to Ion Labs Microbiology Laboratory

5.3 D-1015-F1, Form, Plaque Forming Unit Worksheet

5.4 C-502, SOP, Record Storage, Retention, and Destruction

6.0 Equipment/Materials

6.1 Equipment

6.1.1 Varying Volume Pipettes

6.1.2 Water Bath (42°C)

6.1.3 Shaking Water Bath (37°C)

6.1.4 Varying Weight Scale

6.1.5 Hot/Stir Plate

6.1.6 Incubator (37°C)

6.2 Reagents and Solutions

6.2.1 After media preparation ensure to follow SOP D-113 "Microbiological Media Validation" for growth promotion of the media prepared below before or at time of use.

6.2.2 Luria Bertani (LB) Broth (Storage Temperature 2-8°C)

6.2.2.1 To make 1 L- Dissolve 25g of dehydrated LB Broth in 100mL of DI

Water, follow manufacturer's instructions. Mix with magnetic stir bar on a stir/hot plate to ensure powder is dissolved. Autoclave at 121°C for 15 minutes. Expiration Date 1 month from preparation.

Note: If using previously made broth: check broth for discoloration (discard if broth is turbid). **DO NOT** mix old and new broth batches.

6.2.3 Luria Bertani (LB) Agar (Storage Temperature 2-8°C)

6.2.3.1 To make 1 L-Dissolve 40g of dehydrated LB Agar in 1000mL of DI Water, follow manufacturer's instructions. Mix, with a magnetic stir bar, and heat the solution on a stir/hot plate for a minimum of fifteen minutes. Autoclave at 121°C for 15 minutes. Pour approximately 15 mL of agar into a series of sterile petri dishes. Allow agar to solidify. Expiration 1 week from preparation.

Note: If available, pour plates in a laminar flow hood. Incubate plates at 37°C for 6 hours. If growth is present, discard plates. **Minimize contamination by sterilizing your work surface prior to pouring plates.** Store excess plates in a sealed plate sleeve at 2-8°C.

6.2.4 Luria Bertani (LB) Soft Agar (Storage Temperature 2-8°C)

6.2.4.1 To make 1 L-Dissolve 20 g of dehydrated LB Agar in 1000mL of DI water, follow manufacturer's instructions. Mix, with a magnetic stir bar, and heat the solution on a stir/hot plate for a minimum of 15 minutes. Autoclave at 121°C for 15 minutes. Expiration Date 1 month from preparation.

Note: If using previously made soft agar: check for discoloration (discard if soft agar is turbid). **DO NOT** mix old and new soft agar batches. Warm agar to a liquid state and maintain at 42°C prior to use. Expiration Date 1 month from preparation.

7.0 Procedure

7.1 Safety Information:

7.1.1 Wear gloves and protective eyewear at all times. Avoid inhalation and skin contact of any powdered material as if may cause irritation. Take necessary precaution when dealing with all material.

7.2 Follow instruction closely as time, temps, and RPMs are critical to ensuring plaque formation.

7.3 Microbial-Host Culture

7.3.1 Prepare a TSA plate with QC E. coli a day prior to next step.

7.3.2 Inoculate 1-2 microbial colonies from the TSA plate in a sterile flask containing 25-50 mL of LB Broth, incubate inoculation in a 37°C shaking water bath for approximately 2-6 hours. Set RPMS at 200-300.

7.3.3 Measure the absorbance (600nm) of the inoculated culture against LB broth as a blank. Inoculation absorbance should be between 0.7-1.0 (1.0-1.2 dilute with LB Broth to achieve correct absorption; >1.2 should be discarded).

7.3.3.1 Turn on the Perkin Elmer UV/VIS Lambda 365 instrument and wait 1 hour for it to warm up.

7.3.3.1.1 After instrument has warmed up, open the Perkin Elmer UV WinLab icon on the computer. Log in with username and password.

7.3.3.1.2 Click on manual control, click on the instrument name in the list and press ok.

7.3.3.1.3 Once instrument has completed all its checks, change the wavelength to 600nm.

7.3.3.2 Auto zero the blank (LB Broth) and then take the absorbance of the microbial-host culture.

7.3.4 Store culture at room temperature until use. Expiration date: Daily.

7.4 Sample Preparation

7.4.1 Weigh out an appropriate amount of sample (0.1 g) into a phosphate buffer vial.

7.4.2 Label all phosphate buffer vials with LB number and dilution factors. Targeted dilutions are decided based on the expected activity, sample weight, and should span a dilution above and below a point within the readable range of the assay.

7.4.3 Examples:

7.4.3.1 RMS000726 target activity 2 x 10^8 PFU/g (readable target =20 x 1^7), targeted final dilutions and expected counts should be as follows: 10^6(200 PFU), 10^7(20 PFU), and 10^8(2.0 PFU).

7.4.3.2 SLC00234 target activity 3.0 x 10^6 PFU/g (readable target =30 x 10^5), targeted final dilutions and expected counts should be as follows: 10^4(300 PFU), 10^5(30 PFU), and 10^6(3.0 PFU).

7.4.4 Dilution scheme as follow stopping at the final dilution required for the target activity:

7.4.4.1 Add 100mg (0.1g) of each sample to initial 99ml Phosphate Buffer container, creating a 10^3 dilution. Vortex for 30-60 seconds **(critical time)**. Keep container, do not throw away.

7.4.4.2 Immediately transfer 11 mL of the initial samples dilution into another 99mL phosphate buffer container, creating a 10^4 dilution. Vortex for 30-60 seconds **(critical time)**. Keep container. do not throw away.

7.4.4.3 Immediately transfer 11mL of the second dilution into another 99mL phosphate Buffer container, creating a 10^5 dilution. Vortex for 30-60 seconds **(critical time)**. Keep container do not throw away.

7.4.4.4 Immediately transfer 11mL of the third dilution into another 99mL phosphate Buffer container, creating a 10^6 dilution. Vortex for 30-60 seconds **(critical time)**. Keep container, do not throw away.

7.4.4.5 If more dilutions are needed continue with the 11 mL in 99 mL as needed. (i.e., 10^7,10^8,10^9).

7.5 Assay steps

7.5.1 For each sample, label a minimum of 2 LB agar plates for each target dilution with the following:

7.5.1.1 LB Number

7.5.1.2 Dilution factor (e.g. 10^4, 10^5, 10^6)

7.5.1.3 Date and Tech initials

7.5.2 Reserve one (1) additional LB agar plate for a soft agar control.

7.5.2.1 A soft agar control consists of 100 µL microbial –host culture and 3 mL soft agar.

7.5.3 Reserve one (1) additional LB agar plate for a negative control (blank).

7.5.3.1 A negative control consists of 100 phosphate buffer and 3 mL soft agar.

7.5.4 Incubate LB agar plates at 37°C for a minimum of 30 minutes.

7.5.5 For each sample, prepare a minimum of two (2) test tubes for each target dilution.

7.5.6 Pipette 100µL of each sample into respective test tubes.

7.5.7 Add 100 µL of microbial-host culture to all tubes (except negative control) and vortex for 10 seconds. Incubate at 37°C for a minimum of at least five (5) minutes.

7.5.8 Perform this next step for each tube individually to prevent the soft agar from solidifying before it is in the LB agar plate.

7.5.8.1 Add 3mL of LB soft Agar and immediately but carefully pour onto the respective & appropriately labeled LB agar Plate.

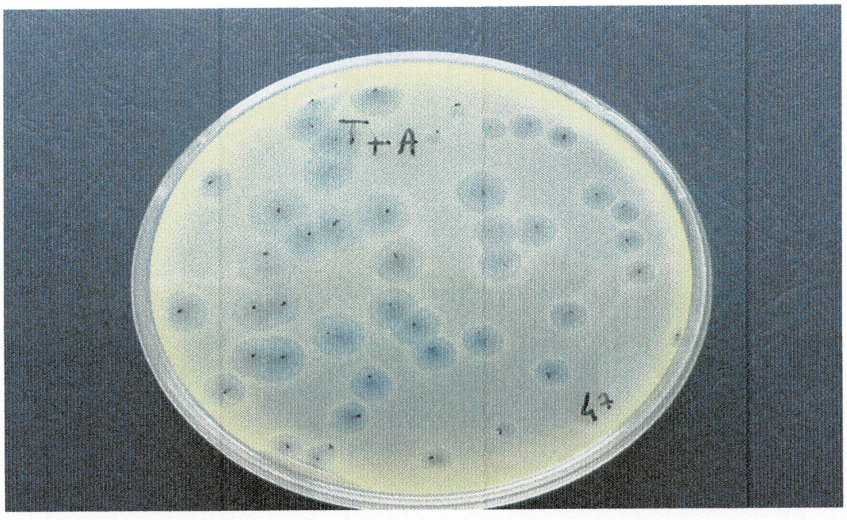
Note: Do not make bubbles, as this will obscure the counting.

7.5.8.2 Incubate the plates at room temperature, agar side down, for approximately 5 minutes (or until soft agar has solidified).

7.5.9 Invert plates and incubate at 37 ± 2°C for a minimum of 12 hours.

7.6 Interpretation

7.6.1 Plaques appear on a bacterial lawn as small clearings on the otherwise homogenous surface of the bacteria lawn (see below).



7.6.2 If the negative bacterial control or soft agar control has visible plaques, cross-contamination has occurred and the procedure must be repeated.

7.6.3 Otherwise, count only those plates having 20-300 plaques, averaging replicates.

7.6.4 If no plate have 20-300 plaques, repeat the procedure at a more appropriate dilution until one or more target dilutions fall within the enumerable range of plaques.

7.6.5 Ideally, the plaque counts from the three sample dilutions should differ by one order of magnitude from its neighbor. If the sets of dilutions do not reflect a 1 log change increasing or decreasing, than an error may have occurred and the procedure must be repeated.

Note: Counts above or below the readable range of the assay may not accurately reflect a 1 log change due to plate crowding or the inherent standard deviation of the assay.

7.7 Calculation

7.7.1 Results, in PFU/g, are determined using the colonies on the media, the amount of sample weighed out and the dilution factor. The dilution factor (DF) is computed as the inverse of the sample concentration.

7.7.2 Example:

7.7.2.1 If a 0.1g sample is diluted into 99mL, with a 11 mL of that solution transferred into another 99mL, followed by another 11 mL of that solution transferred into another 99mL, followed by another 11 mL of that solution transferred into another 99mL, finally followed by 100 µl transferred to the inoculation vial, the dilution factor is calculated as follows:

7.7.2.1.1 DF= $(conc)^{-1}$= (99.1mL) (110) (110) (110) (110) (10) (0.100g) (11.0) (11.0) (11.0) (11.0) 991 x 10 x 10 x 10x 10 x 10= 9.91 $x10^7$

7.7.3 Using the dilution factor, the PFU/g for the sample may be computed using the number of plaques that appear on the plate (N) PFU/g = (DF) N

7.7.4 Example:

7.7.4.1 20 plaques (9.91 $x10^7$) = 1.98 $X10^9$ PFU/g

8.0 Records

8.1 Plaque Forming Unit Worksheet (D-1015-F1) – Fill in all information.

8.2 Records will be maintained following SOP C-502 Record Storage, Retention, and Destruction.

9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	12/12/22	New procedure.	N/A	G. Shaw

10.0 Attachments

N/A

ION LABS	Plaque Forming Unit Worksheet		
	Form: D-1015-F1	CCR No. N/A	Revision: 0

Sample Name / Lot / Formula Number	

E. coli Lot Number/Expiration Date	
LB Broth Lot Number/Expiration Date	
LB Agar Lot Number/Expiration Date	
LB Soft Agar Lot Number/Expiration Date	
37°C Incubator Ion Number/Cal Due	
37°C Shaking Water Bath Ion Number/Cal Due	
42°C Water Bath Ion Number/Cal Due	
Hood Ion Number/Cal Due	
1000 µL pipette Ion Number/Cal Due	
100 µL pipette Ion Number/Cal Due	
Absorbance Analyzer Ion Number/Cal Due	

Absorbance Reading of Microbial-Host Culture	

Dilution	Incubation Start date/time/tech	Incubation End date/time/tech	Plate 1	Plate 2	Average

Calculations: (use only the dilution that is between 20-300 plaques)

DF=

PFU/g=

Completed By: _____ Date: _____

Reviewed By: _____ Date: _____