

## Microbiology for the Health Sciences Laboratory

### Lab Session 9 - Isolation and Titration of Bacteriophages

#### Introduction

Viruses are obligate intracellular parasites of cells. They must, therefore, be grown in cell culture if one wishes to study them in the laboratory. Cell culture of plant or animal tissues is tedious and expensive, which makes study of plant or animal viruses difficult. An alternative is to study bacterial viruses because they can be grown in cells that are easy to prepare and maintain. The purpose of this lab is to grow a purified suspension of virus in a natural host (e.g. an *E. coli* phage in *E. coli*) and then use available techniques to determine the concentration of the virus in the suspension.

#### A. Review the following for reference:

1. Text: Chapter 13 - Pages 305-307 & Fig 13.3c; Pages 310-312 & Fig 13.5; and page 317 & Fig 13.12.
2. Note: Use your best ASEPTIC TECHNIQUES when conducting this lab!

#### B. Do the following during lab:

1. Part 1: Preparation of Phages and *E. coli*

##### Part 1A - Preparation of phage suspension

- a. Add 1000  $\mu$ l (= 1.0 ml) of diluent (sterile water) to a preparation of purified phages (either T1, T2 or T4).  
We could isolate T phages from raw sewage. Instead, we purchase them from the phage store.

##### Part 1B - Dilution of phage suspension. (Techniques, Part 1B)

- a. Add 900  $\mu$ l (= 0.9 ml) of sterile water to each of 7 tubes numbered 1 through 7. Use 'large/blue' micropipet.
- b. Add 100  $\mu$ l (= 0.1 ml) of a phage suspension (from part 1A above) to tube #1. Use 'small/yellow' micropipet. Mix. Discard pipet tip.
- c. Transfer 100  $\mu$ l of the suspension in tube #1 to tube #2. Use 'small/yellow' micropipet. Mix. Discard pipet tip.
- d. Transfer 100  $\mu$ l of the suspension in tube #2 to tube #3. Mix. Discard pipet tip.
- e. Continue this process to tube #7. Use a new pipet tip after every transfer. This is your Phage Dilution Series.

##### Part 1C - Preparation of *E. coli* for infection

- a. Prepare a fresh, actively growing 6 hour culture of *E. coli* in TSB.  
This has already been done.

2. Part 2: Titration of Bacteriophage Suspension

##### Part 2A - Titration using bacterial lawns

We will not do this assay. It usually works, but not well.

##### Part 2B - Titration using a broth clearing assay (Techniques, Part 2B)

We will do this assay. It sometimes works well.

-Preparation of bacterial cell cultures:

- a. Label 8 tubes of TSB 1 through 8.
- b. Add 100  $\mu$ l of *E. coli* to each of the 8 tubes of TSB. Use a single micropipet tip.

- Infection of cells:

- a. Transfer 100  $\mu$ l of phage suspension from tube #7 of dilution series to TSB tube #7.
- b. Transfer 100  $\mu$ l of phage suspension from tube #6 of dilution series to TSB tube #6.
- c. Continue this process to tube #1.

Note: Only one micropipet tip is needed for the entire process!

- d. TSB tube #8 will be a control (i.e. there is *E. coli*, but there is no virus in this tube).
- e. Incubate tubes for ~ 6 hours at 37 C in the rotary shaker.
- f. Record the results in the TSB tubes. Which tubes is there growth or no growth of *E. coli*.

##### Part 2C - Titration using soft/top agar (Techniques, Part 2C)

We will do this assay. It is the standard virus titration assay and it usually works well.

-Preparation of plates:

- a. Label 8 plates of "Bottom Agar" 1 through 8

-Preparation of bacterial cell cultures:

- a. Label 8 tubes of TSA "Soft Agar" 1 through 8
- b. Add 20  $\mu$ l (= 0.02 ml) of *E. coli* to each of the 8 tubes of TSA.

Note: Do not add 0.3 ml as the diagram shows!!!

Note: You must keep the tubes at 50 C or they will solidify, but don't leave the mo's at this temperature long or they will start to die.

Note: You may wish to do the procedure this far and then infect cells one tube at a time.

- Infection of cells:

- a. Transfer 100 ul of phage dilution from Tube #7 and place it in the *E. coli* soft agar tube #7.  
Mix well and immediately pour into Plate #7. Swirl very gently.
- b. Transfer 100 ul of phage dilution from Tube #6 and place in the *E. coli* soft agar tube #6.  
Mix well and immediately pour into Plate #6. Swirl very gently.
- c. Continue this process to plate #1.  
Note: Only two micropipet tips are needed: one for the *E. coli* and one for the phage dilution.
- d. Plate #8 will be a control (i.e. there is *E. coli*, but no virus is added to this plate)
- e. Allow the soft agars to harden. Incubate plates ('bottom down') for 12-24 hours at 37 C.
- f. Record the number of plaques in each plate.

### C. Results:

1. Part 2B: Estimation of phage concentration:
  - a. Note the tube with the greatest phage dilution that inhibited growth of *E. coli*.
  - b. What is the minimum number of phages that are present in the original sample?
2. Part 2C: Calculation of actual phage concentration:
  - a. Count the number of plaques on each plate in Part 2C.  
Note: Only count plaques on plates when there are 30 to 300 plaques total.
  - b. Calculate the number of viruses per ml in the original suspension (Part 1Aa):  
e.g. 48 plaques on plate #3 would =  $48 \times 10^3$  viruses per 0.1ml or 480,000/ml.
  - c. Correlate these data to the results of the broth clearing assay (Part 2B).

### D. Techniques:

The following diagrams describe the dilution series and the experimental approaches.

## E. Calculations, Discussion and Conclusions

Part 2B: Broth Clearing: Record results as growth (+) of phages (i.e. killing of *E. coli*), or no growth (-).

Tube #1	Result _____
Tube #2	Result _____
Tube #3	Result _____
Tube #4	Result _____
Tube #5	Result _____
Tube #6	Result _____
Tube #7	Result _____
Tube #8	Result _____

Minimal number of phages in original sample = \_\_\_\_\_  
Show math.

Part 2C: Plaque Assay: Record number of plaques on plate or TMTC if there are too many plaques to count.

Plate #1	Plaques _____
Plate #2	Plaques _____
Plate #3	Plaques _____
Plate #4	Plaques _____
Plate #5	Plaques _____
Plate #6	Plaques _____
Plate #7	Plaques _____
Plate #8	Plaques _____

Number of phages in original sample = \_\_\_\_\_  
Show math.