

Microbiology Lab Experiment Changes

Experiment #: 6-3

Title: Bacteriophage Plaque Assay

Live Organisms: *E. coli B*, *Bacteriophage T₂* or *T₄*

Changes: Procedure (Work in groups)

1. Soft agar deeps have been melted and are being held at 45 - 50°C in water bath.
2. Use only 4 nutrient broth tubes not 7 as in manual. You are starting with 10⁻⁴ phage dilution. Use only 5 TSA plates.
3. Keep soft agar tubes in little water baths. Perform transfers in the water baths. It is a good idea to put the broth tubes in the water baths also. This way everything is the same temperature.
4. Follow procedure in lab manual except transfer 1.0 mL of each phage dilution to the soft agar deeps **not 0.1mL** as in lab manual and omit the second row of tubes. You will be putting the phage dilution and *E. coli* directly into the soft agar tubes.

Take Home Lesson: Read lab manual for review. Define: plaque, PFU, TNTC, and TFTC. As with the previous serial dilution, the number of plaques must fall between 30 and 300. Calculate the number of pfu's per mL of stock phage culture by multiplying the number of pfu's on a plate times the dilution factor of the phage placed on that plate. What type of phage are we using? What life cycle does it use? Explain the steps in this life cycle.