Microbiology Lab Experiment Changes

Experiment #: 6-4

Title: Plaque Assay of Virus Titer

Live Organisms: *E. coli B, Bacteriophage T_2 or T_4*

Changes: Procedure (Work in groups)

******We are not using microtubes, micropipettes, or microliters!******

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. 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 the procedure on page 2 of this handout. 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.

1. Put all dilution tubes including the original phage tube into water bath.
2. Do all dilutions first and then transfer 1.0mL of each dilution to the corresponding soft agar deep.
3. Then, add 0.2mL *E. coli* to each soft agar deep.
4. Remove soft agar deep from water bath, dry tube with paper towel, then pour each soft agar deep (phage dilution + *E. coli*) on top of an agar plate and <u>immediately</u> gently swirl a few times to spread mixture onto agar surface.
5. Wait 5 minutes before turning agar plates upside down.