Ex 29. BACTERIOPHAGE Plaque Assay

Bacteriophages are viruses which infect bacteria. PHAGE (as in phagocytosis) means "eat", and generally refers to a virus. Most bacteria have phages that are able to parasitize them. In fact, the ability to be infected with a known phage type is used to identify some strains of bacteria (like ***Staphylococcus***); this identification is known as **phage typing** . As the virus infects bacterial cells that it has been mixed with, the lytic infection destroys the bacteria. The bacteria are poured into a Petri plate and forms what is called a bacterial lawn on the agar plate. As the surrounding cells are infected and killed by the released viruses, a clear spot on the agar---in the bacterial lawn---develops, called a **plaque** . The plaques can be counted and the number of virus particles or virions in the original specimen can be quantitated as:

viruses/ml of plaque-forming units/ml

The procedure is really very easy.

- The phage specimen is diluted.
- Bacteria and phage are mixed together in tubes of soft agar.
- The mix is kept warm (in the water bath) until ready to pour over the TSA agar plate
- Pour the mix over the tryptone (TSA) agar plates.

OBJECTIVES:

1. Learn how to culture viruses in a host cell.
2. Quantitate viruses in a specimen.
3. Identify viral plaques in a bacterial lawn.

THE PROCEDURE:

Be SURE to mix the dilutions.
Change pipettes between dilutions

INTERPRETATION

1. Lay the Petri plates right side up, from lowest dilution towards highest dilution.
2. Pick each plate up, hold it up to the light, and determine which one has between 30-300 plaques (you can also use the Quebec colony counters--- it has good backlighting!)
3. Get an accurate count of that plate. Fill in the formula for viral counts.

$$\text{\# viruses (PFUs) /ml} = \frac{\text{\# PFUs}}{\text{dilution (factor) of tube} \times \text{amount (volume) plated}}$$

4. Calculate the number of viruses per ml. of original specimen.

Example of a dilution scheme:

This doesn't match the scheme in the Lab Manual. The volumes and dilution factors are different. It is included here only to 'help clarify' the overall process…