Lab. Class - Bacteriophage

A bacteriophage is a virus that specifically targets bacterial cells. Historically, various aspects of the interaction between viruses and host cells - such as **adsorption, the latent period, burst size (yield), and the manifestation of cytopathic effects** - were first elucidated using phage models. This is due to the cost-effectiveness and rapid replication cycles inherent in these experiments.

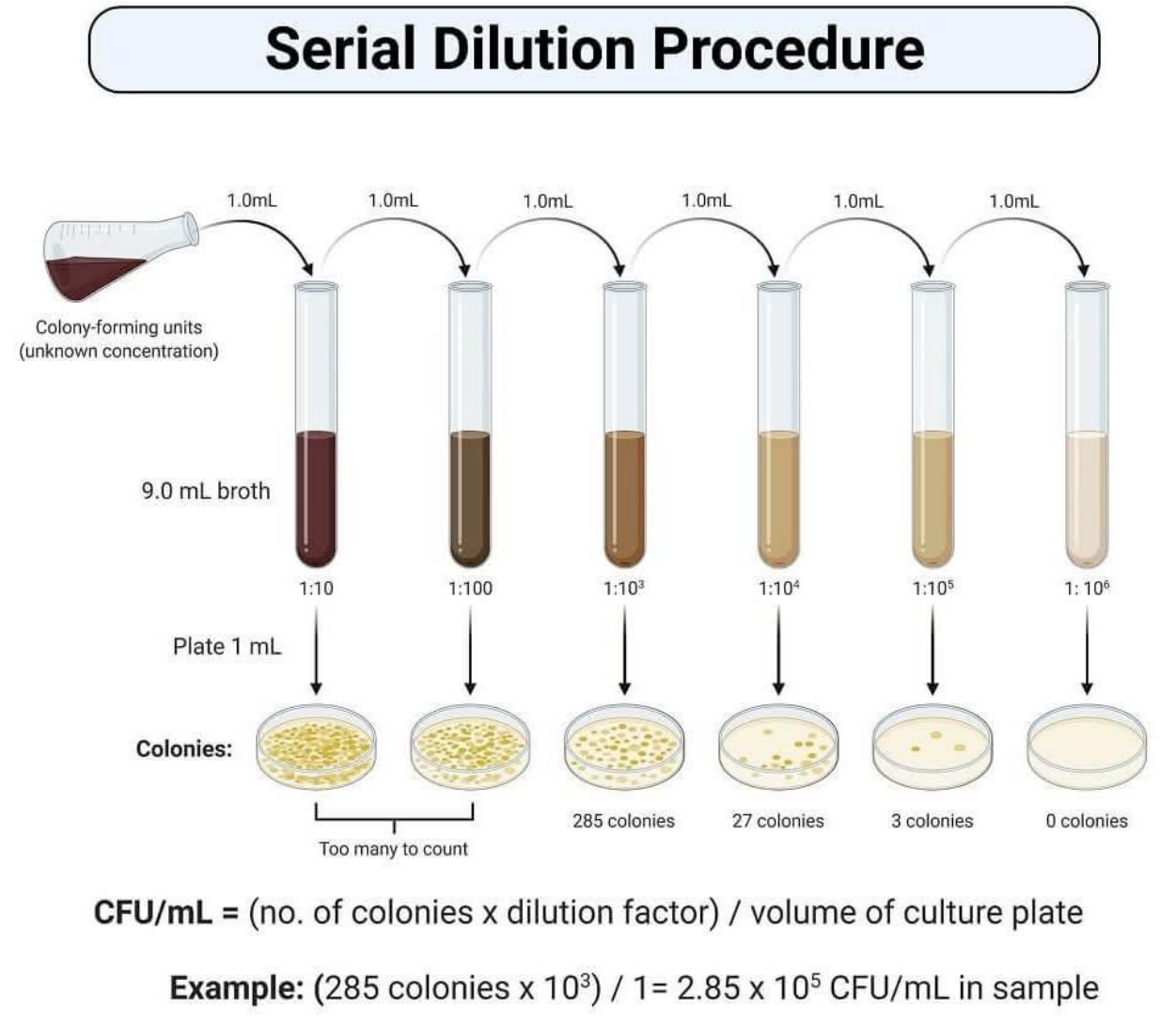
Visualization of Cytopathic Effects. Phage Titration. The Gratia Double-Layer Agar Method

Experimental Procedure:

1. **Materials:** We will utilize a commercial phage cocktail (a polyvalent preparation containing phages active against various microbes). In such preparations, the total phage titer generally does not exceed 10^6PFU/mL.
2. **Serial Dilution:** Prepare six sequentially numbered test tubes, each containing 4.5 mL of sterile physiological saline. Introduce 0.5 mL of the working phage dilution into the first tube and perform a serial dilution through to the sixth tube.

   Note: To ensure accuracy, a fresh pipette tip must be used for each subsequent transfer. This results in a 10-fold reduction in phage concentration at each step.
3. **Inoculation:** Transfer 1 mL from each of the final three tubes (№4, №5, and №6) into new labeled tubes. To each, add 2 drops of a microbial suspension (prepared by washing a 20-hour culture from slant agar with 5 mL of sterile saline).
4. **Plating:** Add melted 0.7% agar to these tubes, shake well, and pour the mixture onto the surface of numbered Petri dishes (containing a base layer of solid agar). Ensure the mixture is distributed evenly across the surface.
5. **Incubation:** Place the plates in an incubator at 37°C.

This technique is formally known as the **Gratia Double-Layer Agar Method**.



Observation and Analysis of Phage Lysis

On the following day, the Petri dishes are examined. **N**egative colonies (plaque) formed as a result of phage replication within bacterial host cells will be clearly visible on the agar surface. Through careful observation, it is possible to differentiate between various phage species based on their unique plaque morphology.

It is important to note a key mechanistic difference: unlike many viruses, a bacteriophage does not enter the target cell in its entirety. Instead, using specialized viral enzymes called **lysins**, the phage penetrates the bacterial cell wall and injects its genetic material (nucleic acid) into the cytoplasm, acting similarly to a molecular syringe. Furthermore, while the **latent period** and viral release in phages typically occur within minutes, human viruses require an average of 10 hours for a single replication cycle.

Determination of the Lytic Spectrum. Spot Tests

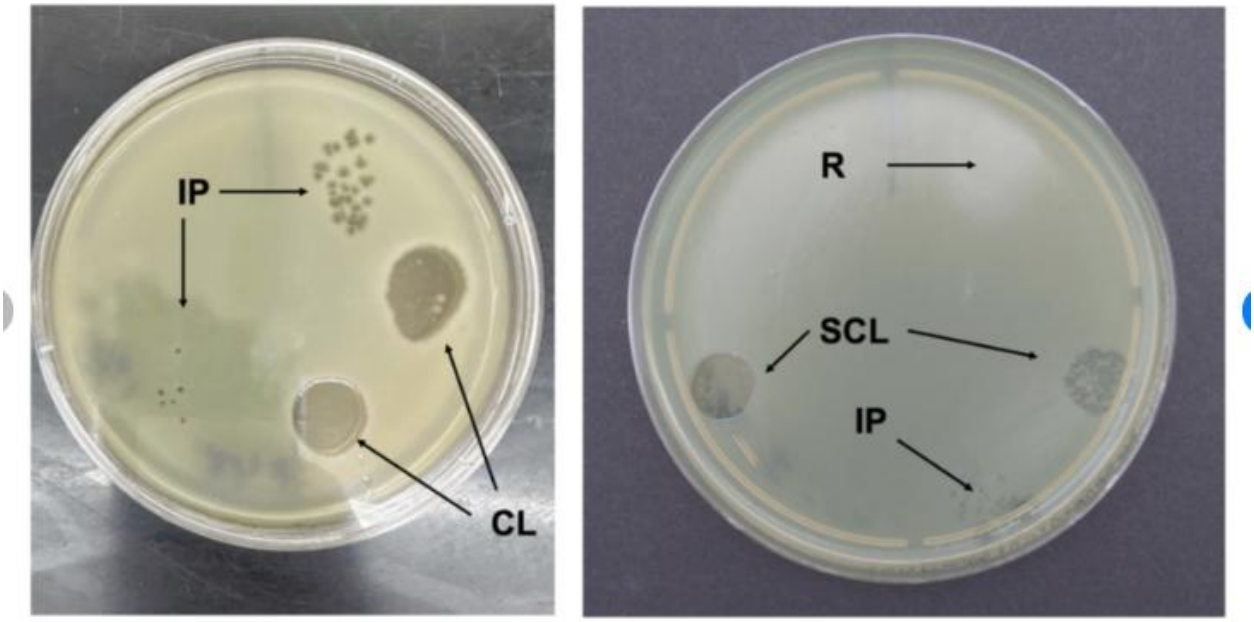
This method also allows the determination of the phage's host range. The lytic spectrum of various phages against a specific bacterium can be determined using the following procedures:

Method A:

A bacterial suspension (prepared as described in the experiment below) is spread across the surface of a nutrient agar plate. After allowing the surface to dry slightly, 20µl aliquots of different phages are spotted onto the lawn. The plates are then incubated for 18–20 hours. Results are recorded the following day based on established phage-sensitivity criteria.

Method B:

1. Prepare 18–20-hour cultures of various bacterial strains and wash them with 5 mL of sterile physiological saline.
2. Using these suspensions, inoculate the agar surface in parallel streaks.
3. Apply a single drop of different phage preparations across the bacterial streaks.
4. Once the droplets have dried, incubate the plates in a thermostat.
5. On the second day, examine the plates for various types of lysis, which are categorized according to the degree of clearing.



Different morphologies of lysis zones by BP on P. aeruginosa growth (Phagogram) are shown: resistant (R), individual plaques (IP), semi confluent lysis (SCL) and clear lysis (CL).

OL - opaque lysis
CL - confluent lysis
SCL - semi-confluent lysis
IP - individual plaques