

# Agar overlay plaque assay

## Aim of the experiment:

This technique is used to produce a homogeneous lawn of bacteria within a thin layer of agar across the surface of a plate. Number of plaque forming units per sample unit volume (pfu/mL) can be calculated. This represents the number of infective particles within the sample which assumes each plaque formed is representative of one infective virus particle.

## Materials

- 1 % Agarose NZCYM Medium plates
- 0.5 % Agarose NZCYM Medium
- O/N culture of host bacterium
- NZCYM Medium
- 10-fold dilutions of phage stock
- 1 x PBS
- 1 mM  $\text{MgCl}_2$  and  $\text{MgSO}_4$
- Sterile 15 ml Falcons Tubes

## Procedure

1. Melt 0.5 % Agarose NZCYM Medium until liquid.
2. Prepare 4 ml of liquid 0.5 % Agarose NZCYM Medium in a 15 ml falcon tubes for each plate.
3. Place 15 ml falcon tubes in the water bath at 48°C.
4. Prepare dilution row of phage solution.
5. Add 100  $\mu\text{l}$  of phage dilution row and 100  $\mu\text{l}$  O/N culture of host bacterium to pre-warmed 0.5 % Agarose NZCYM Medium.
6. Mix the liquid agar/bacterial/phage suspension and pour it evenly across the top of the 1 % NZCYM agar plate.
7. Let the suspension solidify for 5-15 minutes.
8. Incubate plates at 37°C for an appropriate time (min. 2 hours, max. O/N).
9. Count formed plaques.
10. Calculate  $\text{PFU/mL} = \text{Plaque number} \times V (\text{Phage solution}) \times \text{dilution factor}$ .