

## PROCEDURE FOR PHAGE INHIBITION

(from Shraga Segal)

1. Dilute sera in P & G buffer to appropriate concentrations. ( $10^{-4}$  -  $10^{-7}$ ).
2. Place 0.1 ml sera into 13 x 100 tube. Add 0.1 ml of T<sub>4</sub> phage ( $2.5 \times 10^{-5}$ ).  
For each run 4 controls without any sera must be used as a reference for the 0 percent inhibition points.
3. Incubate for 2-1/2 hours.
4. Toward end of incubation heat soft agar (in a double boiler) and dispense in 2.5 ml aliquots. Keep these at 48°C.
5. Just prior to end of incubation period, add 0.2 ml E. coli B harvested at mid log phase to about 20 agar tubes. NOTE: The E. coli will die if left in the 48°C bath too long.
6. At the end of the incubation period, mix the E. coli-agar with the phage-sera and immediately pour onto agar plates with agitation.
7. Incubate for ~14 hours at 37°C.
8. Computations are made as a percent inhibition of the controls.
9. Each sera should be run in at least duplicate.