

Hemagglutination Technique

1. All sera to be titered is inactivated at 56°C for 30'.
2. Diluent: 1X PBS + .2% (w/v) BSA
or Kolmer's Saline + 0.2% BSA if doing hemolysin titers.
Kolmer's saline = 100 mg $MgCl_2$ + 40 mg $CaCl_2$ per liter of normal saline (0.9%)
3. Add 25 λ of diluent to each well (U-bottom microtiter plates-Cooke Engineering Co) using 25 λ dropper
4. Add 25 λ of desired dilution of serum to first well
5. Make consecutive 1:2 dilutions of original solution using titering loops.
Loops hold 25 λ of liquid
Before use - flame over bunsen and plunge into normal saline-allow to cool
Insert loop into 1st well and spin relatively slowly about 5-10 times
pick up loop and insert into 2nd well - spin
pick up loop and insert into 3rd well etc,etc.
After 12th well the remaining 25 λ In loop is drawn out by couching tip to clean diaper.
Loops are then rinsed 5-6 times by dipping in saline and drawing out liquid on diaper
6. Add 25 λ of 2% SRBC suspension using 25 λ dropper.
7. Mix plate by agitating it between fingers- holding it flat on the table top.
8. Allow \sim 1-1 1/2 hrs settling time - read
9. If doing hemolysis - add 25 λ of C' at 1/10 in Kolmer's saline to each well
after sera are titrated and before or after addition of SRBC.