



Medical laboratory techniques
(Viruses Practical -Lab 7-8)
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The complement fixation

Complements are special chemicals or protein components that are a part of our humoral immunity and aid in establishing antigen-antibody complexes, engulfing, degrading, and washing them away. They are found in the blood or attached near membranes. Eg. C1, C2, C3, C4 proteins, etc.

The complement system is a collection of several proteins which interact with each other to initiate a series of reactions to assist in the defense of our immune system.

The complement fixation test:

is based on the principle that the Ag-Ab complex can only fix the complement and its effect on the hemolysis of RBC used in the indicator system. If the sample contains desired antibodies or antigens, the Ag-Ab complex will be formed in the sample after the addition of a complementary reactant (antigen or antibody, based on the component being detected), and the indicator system will not be able to react to the added complement (as it already gets fixed with Ag-Ab complex) which results in no change in the indicator system.

Complement Fixation may be used for :

- Adenovirus
- Fungal Panel (Blastomyces, Coccidioides, & Histoplasma)
- Influenza A & B
- Para influenza 1, 2, & 3
- Poliovirus 1, 2, & 3
- Respiratory Syncytial Virus (RSV)

Advantages:

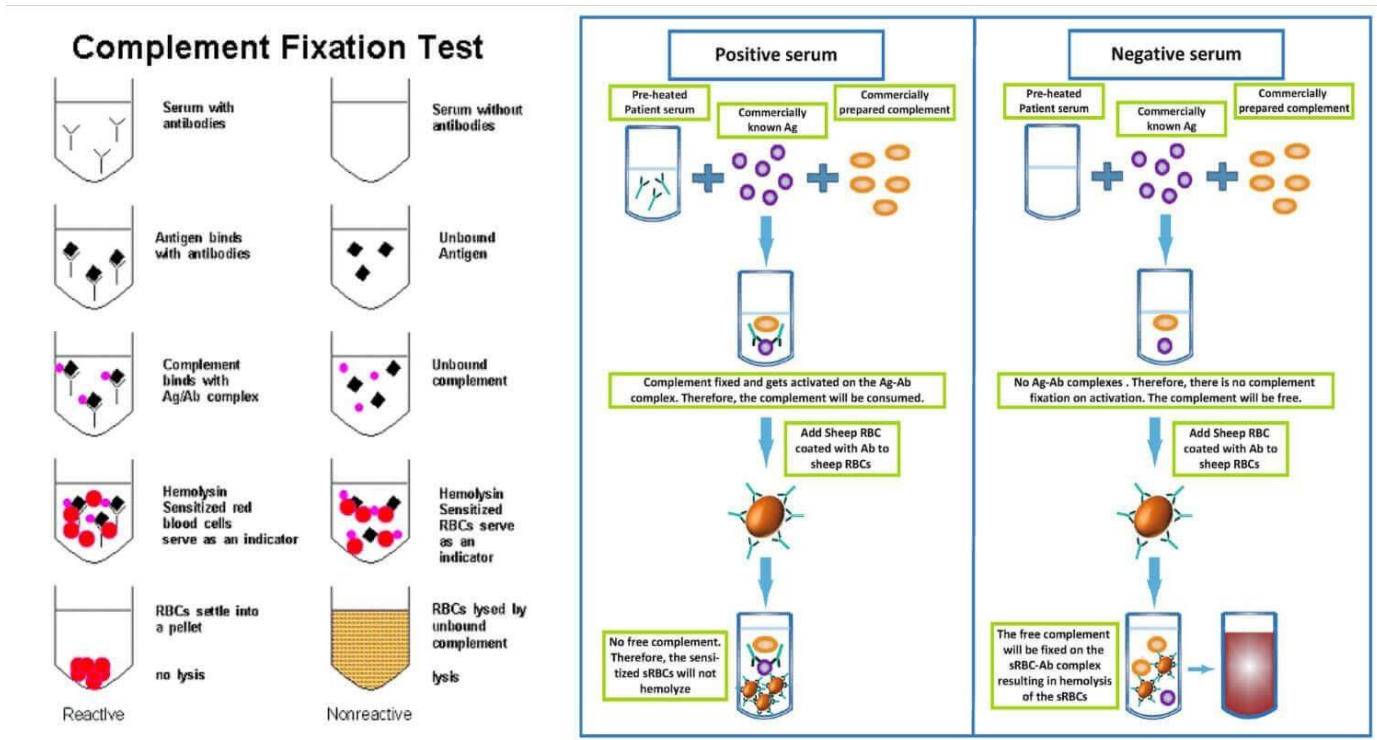
- Easily interpretation results .
- It can be used for the detection of a very small number of antigen or antibody components in the sample.
- It can be used for the detection of a variety of infections.
- It has good sensitivity

Procedure:

1. A known antigen (Viral Ag) is mixed with inactivated patient's serum
2. Add a measured amount of complement (Guineapig serum) in the test system
3. The test system is incubated at 37°C for about 1 hour.
4. After 1 hour an indicator system (sensitized RBC) is added to the test system and again incubated at 37°C for 30 minutes
5. If Ag and Ab matches, they form Ag-Ab complex and utilizes complement.
6. Observe the result

***If hemolysis is observed:** it indicates the absence of specific antibody in patient serum, so that complement has not been used which lysed the sensitized RBC giving hemolysis.

***If no hemolysis is observed:** it indicates that the patient serum contains antibody which reacts with Ag to form Ag-Ab complex and then fix complement .so that no complement is available to hemolyse sensitized RBC.





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Neutralization test (Nt) for virus diagnosis

The neutralization test: is a serological method used to detect the presence of antibodies that prevent of a virus infection. is a highly sensitive and specific test that may be applied to influenza A viruses (IAV) in swine it requires cell culture, more time and labor. The virus neutralization test is important because it can be used to help determine the prevalence of a disease, the geographic dissemination of its virus

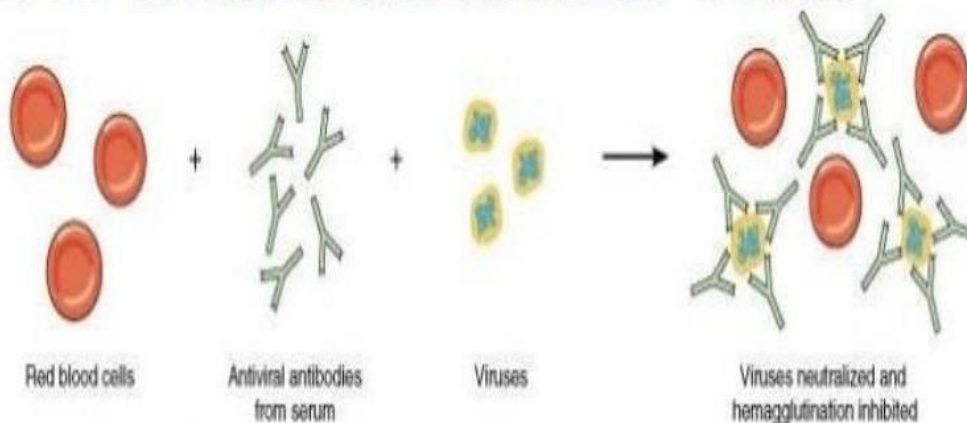
The principles of neutralization test:

To test for virus neutralization, virus and serum are mixed together, incubated under appropriate conditions, and then inoculated into a susceptible living host for detection of nonneutralized virus. Nonneutralized virus is detected by looking for viral growth using indicators such as cytopathic effect (CPE), or metabolic inhibition.

Uses of Neutralization test:

- 1- for virus diagnoses infection based on the host's immune response.
- 2- a research tool for dissecting how antibodies protect the host from infection

Virus Neutralization Tests



Procedure:

- 1-make dilution of serum (antibody)
- 2-a fixed amount of virus is add to each dilution.
- 3-the reaction is incubated for 1hour to allow antibody to bind to and inactivate virus.
- 4-each reaction mixture is inoculated onto indicator cell culture to determine if any infected virus remains.
- 5-the cell culture incubate for few day to allow any virus to grow, then fixed and stained
- 6- the highest dilution of serum that neutralies all the virus . the antibody titer is taken as the reciprocal of the dilution.
- 7-for example if a serum dilution of 1:128 neutralised the virus the antibody titer of that serum is said to be 128

