IMMUNOLOGY

Complement fixation test

Third Stage

Introduction:

The complement fixation test is an immunological medical test that can be used to detect the presence of either specific antibody or specific antigen in a patient's serum, based on whether complement fixation occurs.

Complement Fixation Test Requirements:

- **Samples** such as serum or CSF (may or may not contain the specific antigens or antibodies of interest)
- **Complement Proteins:** The native complement present in the sample is inactivated. Complement obtained from the serum of other organisms such as Guinea pig is added to the sample during the test.
- **Indicator System:** Sheep erythrocytes or RBCs coated with antibodies (mainly derived from Rabbit serum) on the surface. These RBCs can also be called sensitized RBCs.

Complement Fixation Test Principle

Positive principle:

Antibody in sample + Antigen (added) + Complement → Ag-Ab Complex Fixed with Complement

→ Complement fixed Ag-Ab + Indicator System (sensitized RBC) → No change (No hemolysis).

Negative principle:

Sample with no antibody + Antigen (added) + Complement → Antigen + Free Complement

Antigen + Free Complement + Antibody in indicator system (on RBC) → Ag-Ab complex + Complement → Hemolysis

Procedure of complement fixation test:

1. A known antigen (cardiolipin/Viral Ag/sheep RBC) is mixed with inactivated patient's serum
2. Add a measured amount of complement (Guinea pig 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 match, they form Ag-Ab complex and utilizes complement.
6. Observe the result

- Positive result: **No change (No hemolysis)**
- Negative result: **Hemolysis**