DEPARTMENT OF BIOTECHNOLOGY

- “Complement Fixation Test”

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COMPLEMENT FIXATION TESTS

Introduction:

- Complement is a protein (globulin) present in normal serum.
- Whole complement system is made up of nine components: C1 to C9
- Complement proteins are heat labile and are destroyed by heating at 56°C for 20 – 30 min in a process called heat inactivation.
- Complement binds to Ag-Ab complex
- When the Ag is complexed with Ab on surface of cell ,Complement causes lysis of cell.

In general, complement fixation tests (CFT) are best performed in reference laboratories where facilities exist for the careful standardization and control of reagents, which these tests require.

Principle of (CFT)

The complement fixation tests is a technique that has been used over many years to detect and quantify antibody that Serology does not agglutinate or precipitate when reacted with its antigen, but can be demonstrated by its use, or fixation, of complement.

Antigen-antibody reactions lead to immune complex formation that produces complement fixation via the classical pathway. That is when complement takes part in antigen antibody reactions; it is bound or fixed to the antigen antibody complexes. When these complexes are on bacteria, red cells or other cells, the complement brings about the lysis of the cells involved.

This may be exploited to determine the amount of antigen or antibody present in the patient sample. Complement fixation test can detect antibody at a level of less than one microgram per milliliter.

COMPLEMENT FIXATION TEST

- The Complement system is a part of the immune system that **enhances (complements) the ability of antibodies and phagocytic cells** to clear microbes and damaged cells from an organism, promotes inflammation, and attacks the pathogen's cell membrane.
- It is part of the **Innate immune system**.
- The Complement fixation test (CFT) is one of the major traditional tests for the **demonstration of presence of specific antigens or antibodies**.
- CFT was extensively used in Syphilis serology after being introduced by **Wasserman in 1909**.
- CFT consist of **2 steps.**

First step – Complement fixation stage

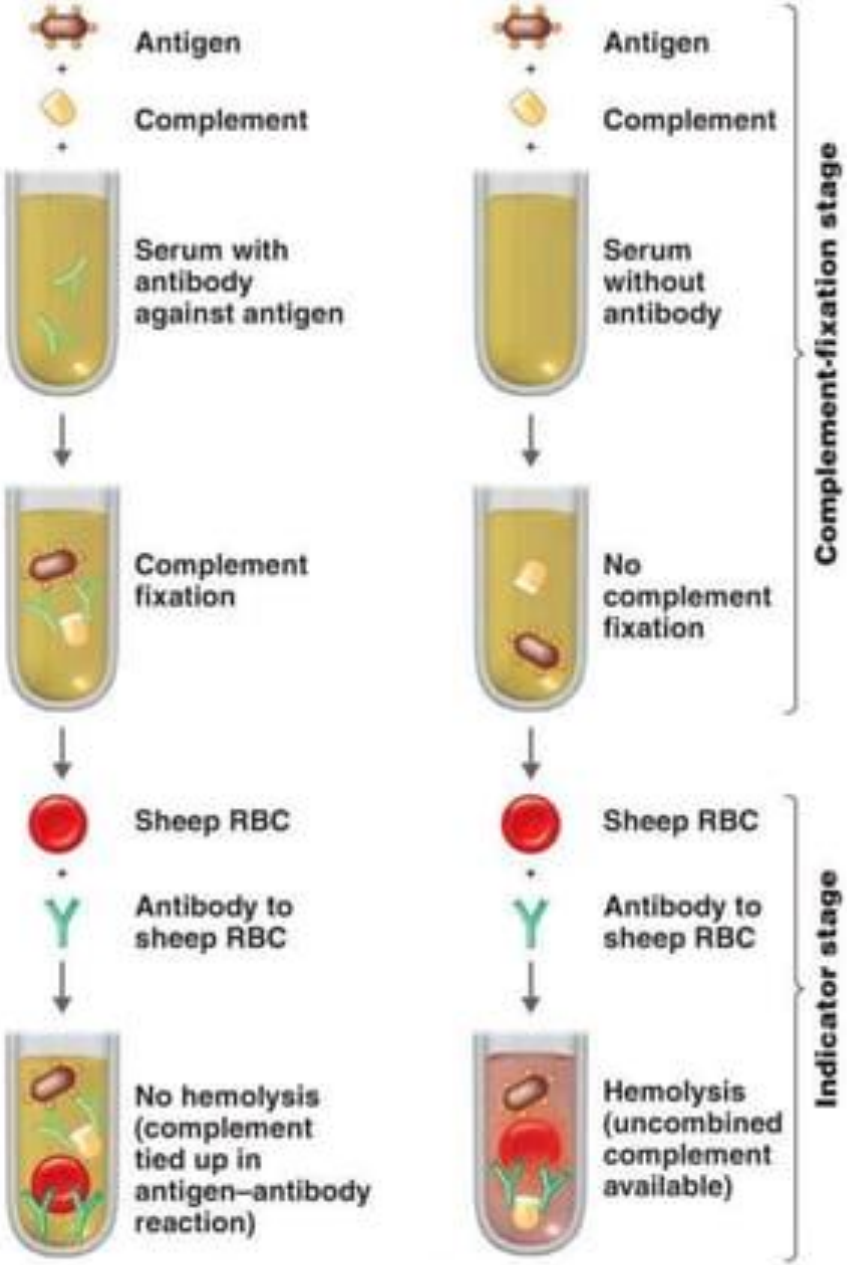
- A **known antigen and inactivated patient's serum are incubated** with a standardized, **limited amount of Complement** (Patient's serum is heated at 56 °C for 30 minutes to inactivate endogenous complement which may disturb the test calibration).
- If the serum **contains specific**, **complement activating antibody** the complement will be activated or fixed by the **Antigen-antibody complex**.
- If there is **no antibody** in the patient's serum, there will be **no formation of antigen-antibody complex**, and therefore complement will not be fixed.

Second step – Indicator Stage

- The second step detects whether complement has been **utilized in the first step or not**. This is done by adding the **Indicator system**.
- If the Complement is fixed in the first step due to the presence of antibody in the patient's serum **there will be no complement left to fix to the indicator system**.
- If there is no specific antibody in the patient's serum, there will be no antigen-antibody complex, and therefore, complement will be present free or unfixed in the mixture. This unfixed complement will now react with the **Antibody - coated sheep RBCs** to bring about their lysis.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.



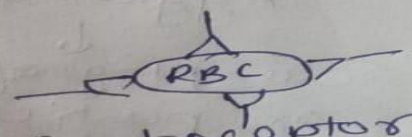
(a) Positive test. All available complement is fixed

(b) Negative test. No antigen–antibody reaction

Patient Serum Heat at 56°C. If complement protein not it affect test. To inactivate

complement synthetic
↓
taken from guinipig
↓
Ag + Ab complex
↓
complement protein attacks
↓
Complement protein fix
↓
To vaiv y
↓
Use Indicator / Amboceptor

RBC (Sheep) $\xrightarrow{\text{Inject}}$ Rabbit
↓
Ab developed
Y Y Y
Anti RBC Ab'S



Amboceptor
↓
Add in TT
↓
No free complement because it is fixed
↓
can not cause the lysis of RBC

Results

- **Positive test** : The available complement is fixed by Ag-Ab complex and No hemolysis of sheep RBCs occurs. So the test is positive for presence of antibodies.
- **Negative test**: No Ag-Ab reaction occurs and the complement is free. This free complement binds to the complex of sheep RBC and it's antibody to cause Hemolysis, causing the development of pink color.

Advantages

- Ability to screen against a large number of **viral and bacterial infections at the same time**.
- Economical.

Disadvantages

- Not sensitive - cannot be used for immunity screening.
- Time consuming and labor intensive.
- Often non-specific e.g. **Cross - reactivity** between Herpes Simplex Virus and Varicella Zoster Virus.

THANK YOU