Antigen – Antibody

Reaction (3)

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Learning objectives:

- introduction to Antigen Antibody reactions.
- **Antigen Antibody reactions part1:** Precipitation, Floccula tion and Immunodiffusion.
- **Antigen Antibody reactions part 2:** Agglutination.
- **Antigen Antibody reactions part 3:** Complement Fixation Test and Neutralization test.

Complement Fixation Test

CFT

complement fixation test

- is the reaction that used complement proteins in fixation of antigen-antibody complex.

- This process of complement fixation can be used to detect very specific and small amount of antibody in the patient's serum. Antibodies that do not produce a visible reaction, such as in precipitation or agglutination, can be demonstrated by fixing of complement during of antigen-antibody reaction.

- Complement-fixation can be used in diagnosis of syphilis (Wasserman test in1909) and still used to diagnosis certain viral, fungal, and rickettsial diseases.

Principle of CFT

- Complement usually not bind to free antibody or free antigen.
- AG-Ab complex fix complement : complement only bind to **bound Antibody** (Ab attached to antigen) at **Fc protion.**

This test is based on the use of complement, a Biologically labile serum factor that causes **the immune cytolysis** i.e. lysis of antibody coated cells

The complement fixation test is performed in two steps :

1. Complement fixation
2. Indicator step

Materials and Reagents

The test requires five reagents and is carried out in two steps.

Test System

- **Antigen:** It may be soluble or particulate.
- **Antibody:** Human serum (May or may not contain Antibody towards specific Antigen)
- **Complement:** It is pooled serum obtained from 4 to 5 guinea pigs. It should be fresh or specially preserved as the complement activity is heat labile (stored at -30 °C in small fractions). The complement activity should be initially standardized before using in the test.

Indicator System (Haemolytic system)

- **Erythrocytes:** Sheep RBC
- **Amboceptor (Hemolysin):** Rabbit antibody to sheep red cells prepared by inoculating sheep erythrocytes into rabbit under standard immunization protocol.

Complement fixation stage

First step

01

A known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement.

02

patient's serum is heated at 56°C for 30 minutes to inactivate endogenous complement which may disturb the test calibration.

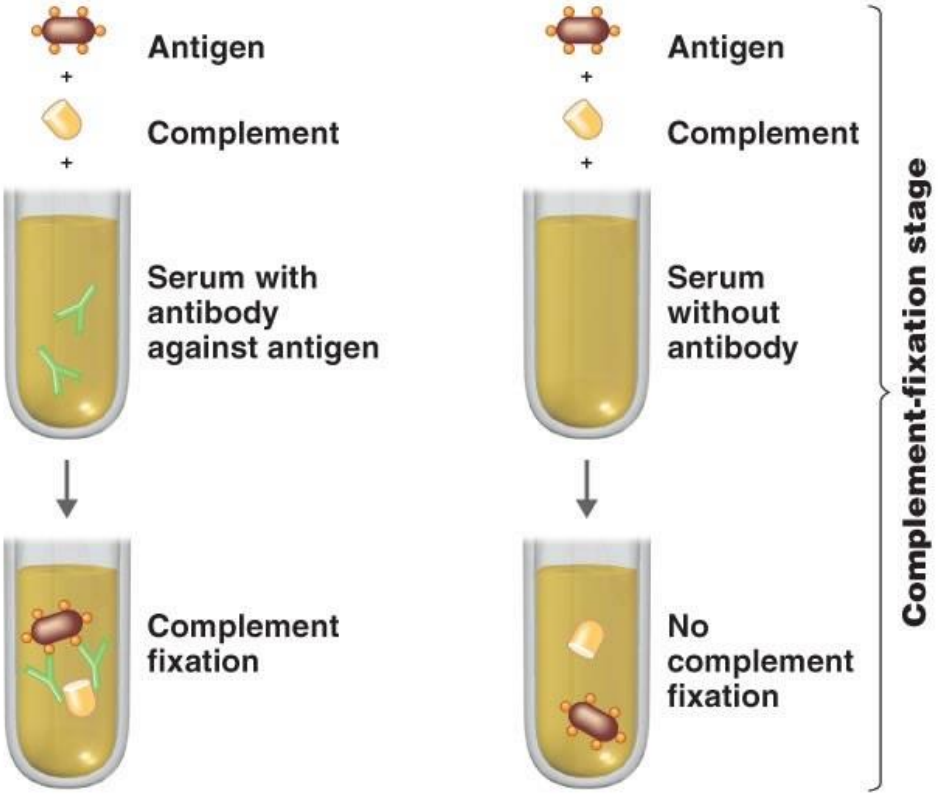
03

If the serum contains specific complement activating antibody, the complement will be activated or fixed by the antigen-antibody complex.

04

However, if there is no antibody in the patient's serum, there will be no formation of antigen-antibody complex, thus complement will not be fixed but **will remain free** (In the indicator stage this complement will react with RBC coated with antibody to sheep RBC).

Complement fixation stage



Indicator Stage

Second step

01

The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system.

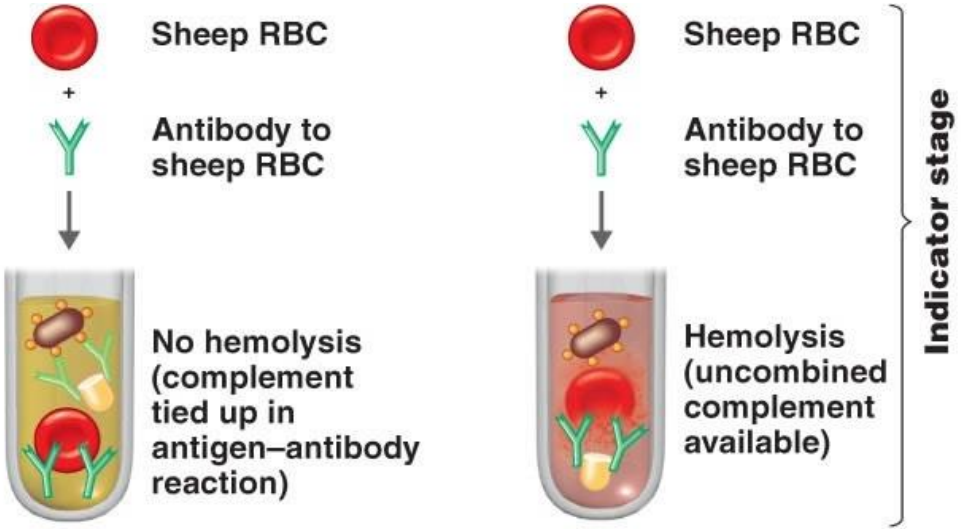
02

If the complement is fixed in the first step owing to the presence of antibody there will be no complement left to fix to the indicator system. There won’t be any lysis of RBCs

03

However, if there is no specific antibody in the patient’s serum, there will be no antigen-antibody complex, therefore, complement will be present free or unfixed in the mixture. This unfixed complement will now react with the anti body- coated sheep RBCs to bring about their lysis.

Complement fixation stage



(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.

(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.

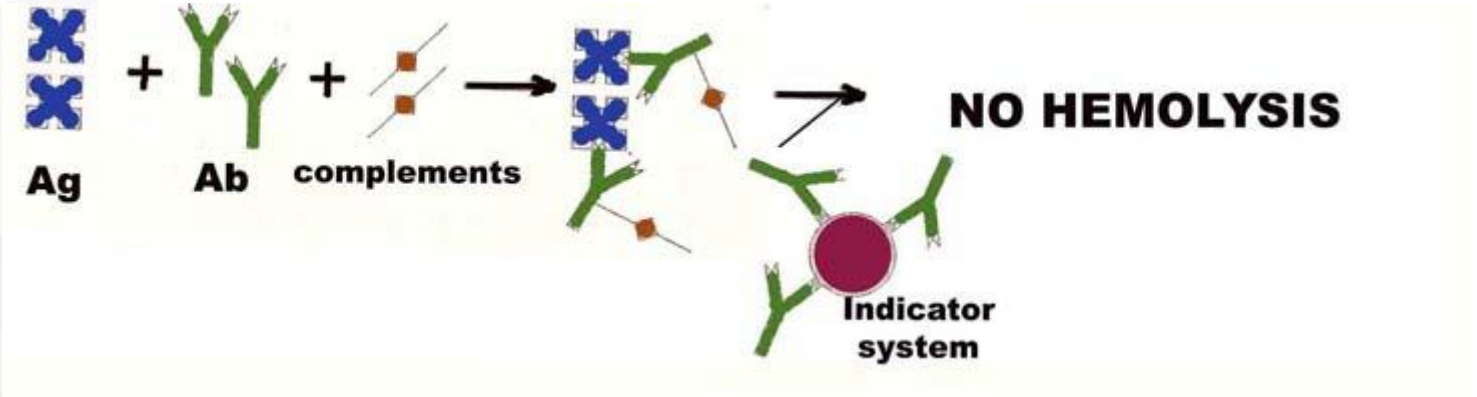
Positive Test

- Step 1:

Antigen + **Antibody** + **Complement** (**from serum**) —At 37°C, 1 Hour→ Complement gets fixed

- Step 2:

Fixed Complement complex + Haemolytic system —At 37°C, 1 Hour→ No haemolysis



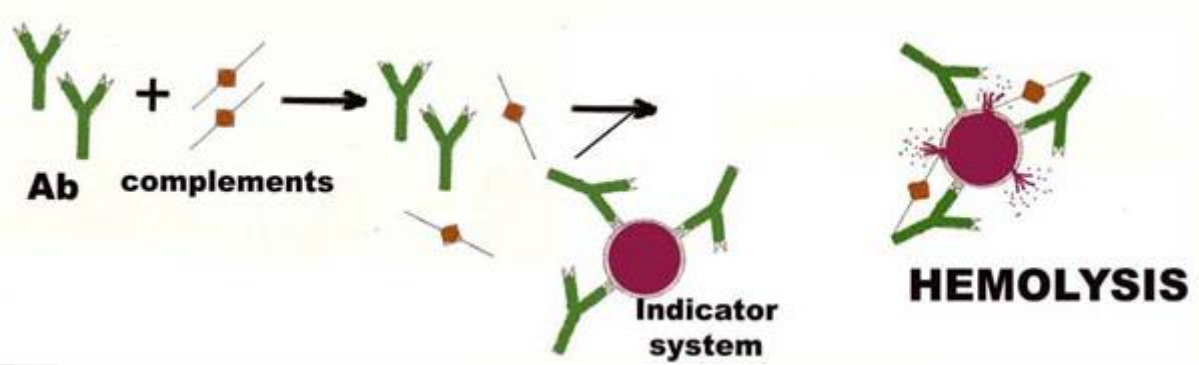
Negative Test

- **Step 1:**

Antigen + **Antibody absent** + **Complement** $\xrightarrow[1\ \text{Hour}]{\text{At } 37^\circ\text{C}}$ Complement **not** fixed

- **Step 2:**

Free Complement + Haemolytic system $\xrightarrow[1\ \text{Hour}]{\text{At } 37^\circ\text{C}}$ Haemolysis



Interpretation

+

In the 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.

-

In the negative test : No Ag-Ab reaction occurs and the com plement 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.

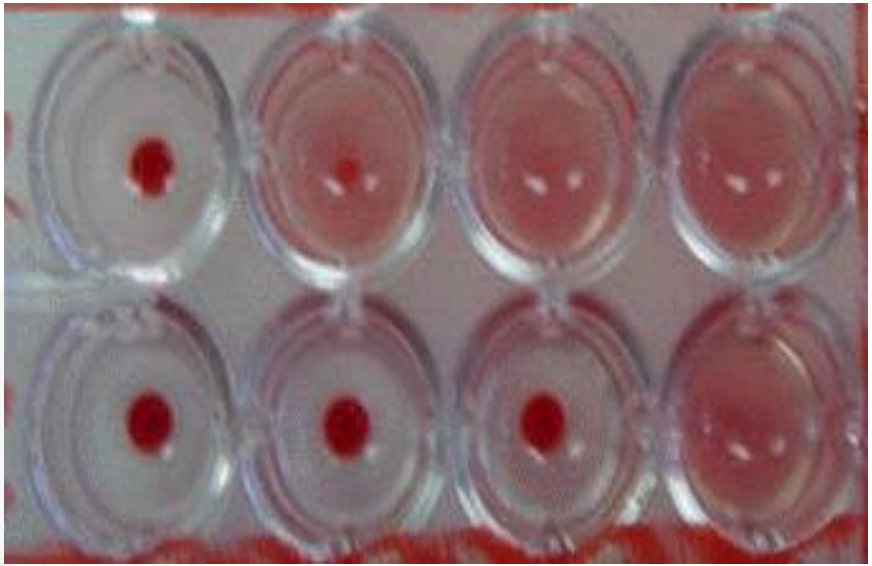
C

Controls should be used along with the test to ensure that

- Antigen and serum are not anti complimentary
- The appropriate amount of complement is used and

The sheep red blood cells do not undergo autolysis

Results and Interpretations:



	1	2	3	4
A	POSITIVE No haemolysis	POSITIVE No haemolysis	NEGATIVE Haemolysis	NEGATIVE Haemolysis
B	POSITIVE No haemolysis	POSITIVE No haemolysis	POSITIVE No haemolysis	NEGATIVE Haemolysis

Advantages and disadvantages of CFT

Advantages

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

Disadvantages

1. Not sensitive - cannot be used for immunity screening
2. Time consuming and labor intensive
3. Often non-specific e.g. cross-reactivity between HSV and VZV

Neutralization test

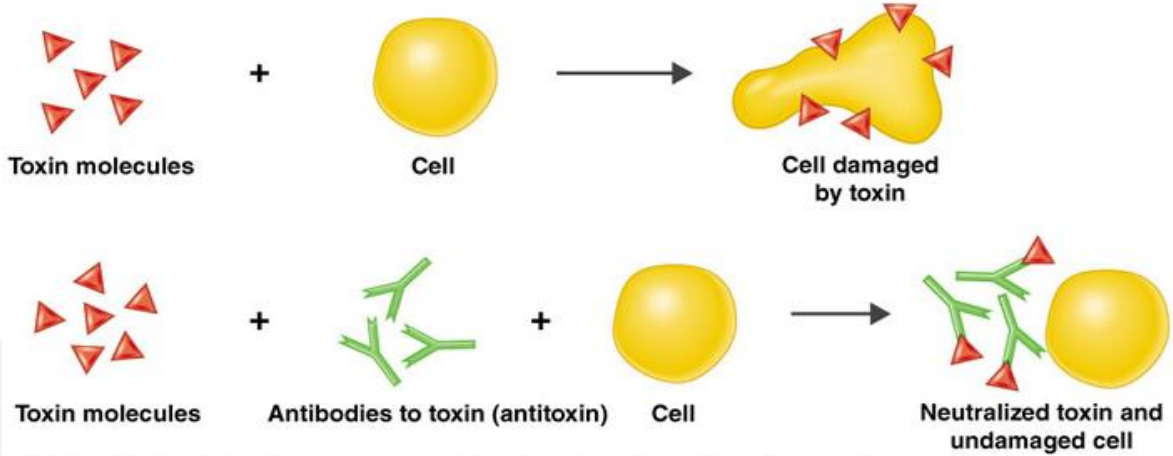
Neutralization

- it is an antigen-antibody reaction in which the harmful effects of a bacterial exotoxin or a virus are blocked by specific antibo dies.

- For example when the serum antibody neutralize the toxic sub stances produced by the diphtheria pathogen, *Corynebacterium diphtheriae*. Such as a neutralizing substance, which is called an “Antitoxin”? .

Neutralization

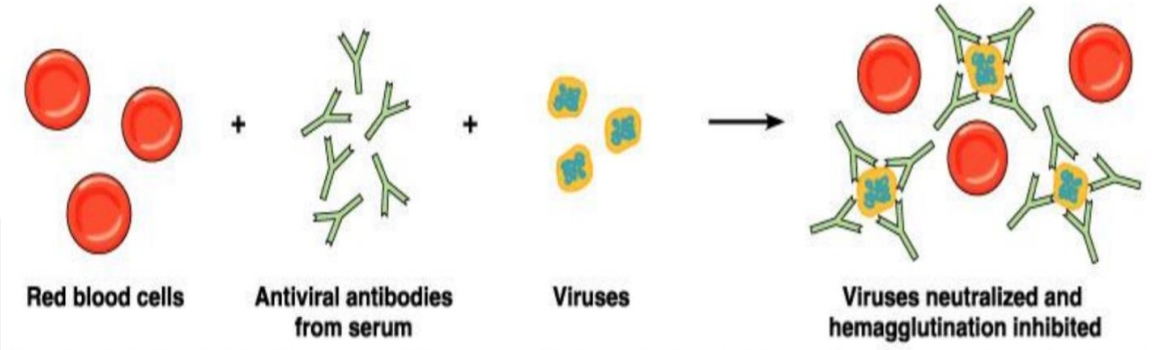
Antitoxin: is a specific antibody produced by a host as it respond to a bacterial exotoxin or its corresponding toxoid (inactivated). So the antitoxin combines with the exotoxin to neutralize it.



(a) The effects of a toxin on a susceptible cell and neutralization of the toxin by antitoxin

Viral hemagglutination inhibition test

- It is one of most frequently used neutralization test
- The test is used in the diagnosis of influenza, measles mumps, and a number of other infections caused by viruses that can agglutinate red blood cells.
- If the person's serum contains antibodies against these viruses, these antibodies will react with the viruses and neutralize them.



(b) Viral hemagglutination test to detect antibodies to a virus. These viruses will normally cause hemagglutination when mixed with red blood cells. If antibodies to the virus are present, as shown here, they neutralize and inhibit hemagglutination.

Thank you

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