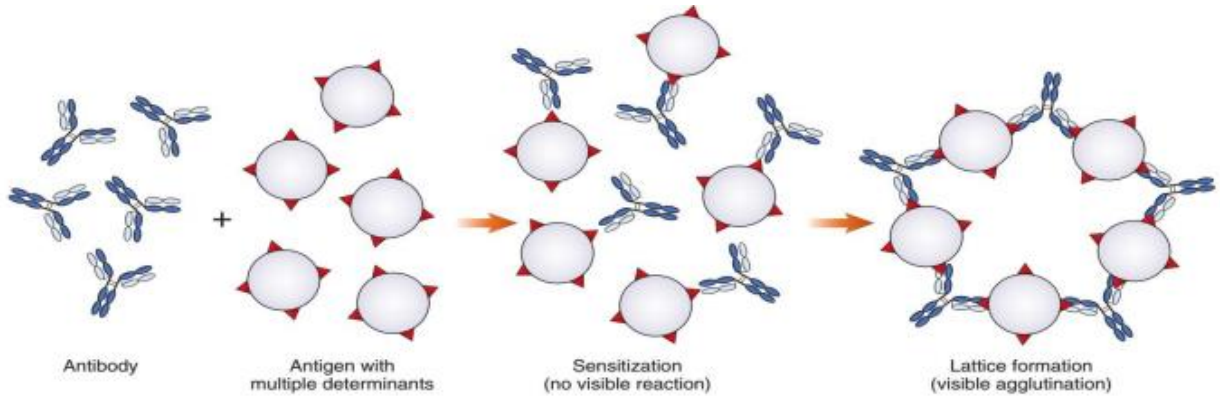
I- Agglutination and Hemagglutination Reaction: Coombs Test

1. Principle of Agglutination:

When the antigen is present on the surface of a large particle, antibodies can induce its agglutination. This process is particularly easy to carry out with bacteria and erythrocytes, the latter of which can be used directly or as carriers for soluble antigens fixed to their surface (passive hemagglutination). This principle is commonly used for blood group determination and is known as the direct hemagglutination reaction.

In this case, agglutination is induced by incubating anti-A or anti-B antibodies with the red blood cells of the subject to be typed. If the subject has antigen A on the surface of their red blood cells (group A patient), agglutination will be observed with anti-A antibodies but not with anti-B antibodies.



2. The Different Agglutination Techniques

Agglutination techniques can be active (direct) or passive (indirect).

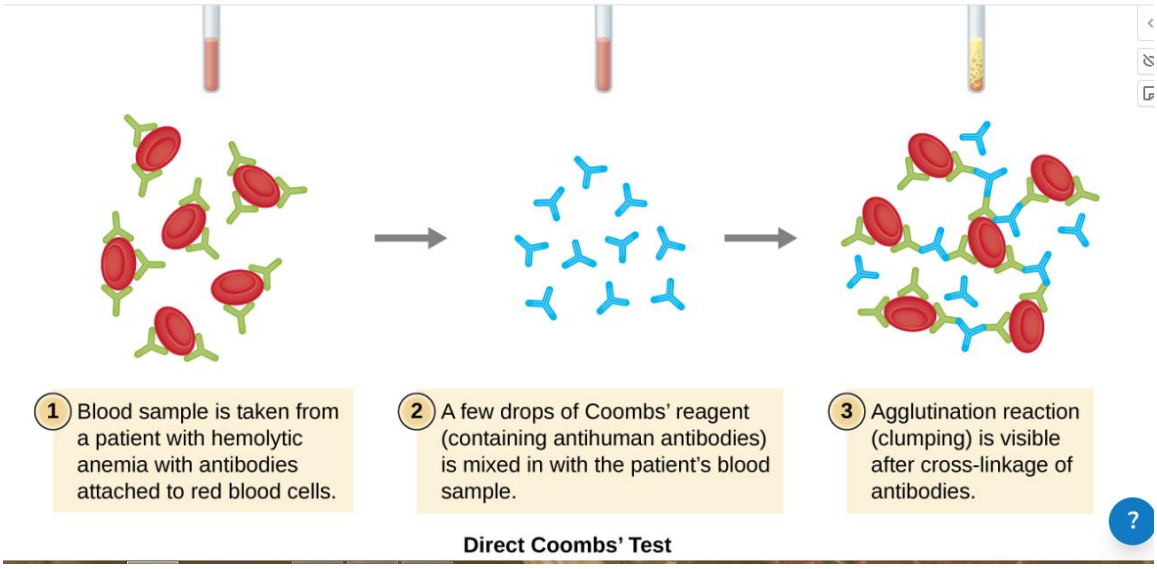
a. "Direct" or "Active" Agglutination Techniques:

"Active" or "direct" agglutination techniques can only be used when the antigen is part of the agglutinated particle (for example, an antigen located on the external surface of a cell or bacterial membrane) and is directly accessible by the antibody.

Examples of techniques: ABO blood typing, the direct Coombs test.

- **Blood typing:** The subject's red blood cells are mixed with anti-A, anti-B, and anti-AB antibodies. Depending on the antigens of the subject's blood group, the red blood cells agglutinate in the presence of the corresponding antibody.

- **Direct Coombs test**: The patient's red blood cells are used in the presence of agglutinating anti-immunoglobulin antibodies (antiglobulin test). A positive agglutination indicates that the patient's red blood cells have already bound pathogenic antibodies (this is the case in autoimmune hemolytic anemia caused by autoantibodies against erythrocytes or in hemolytic disease of the newborn caused by maternal anti-Rh antibodies, for example).

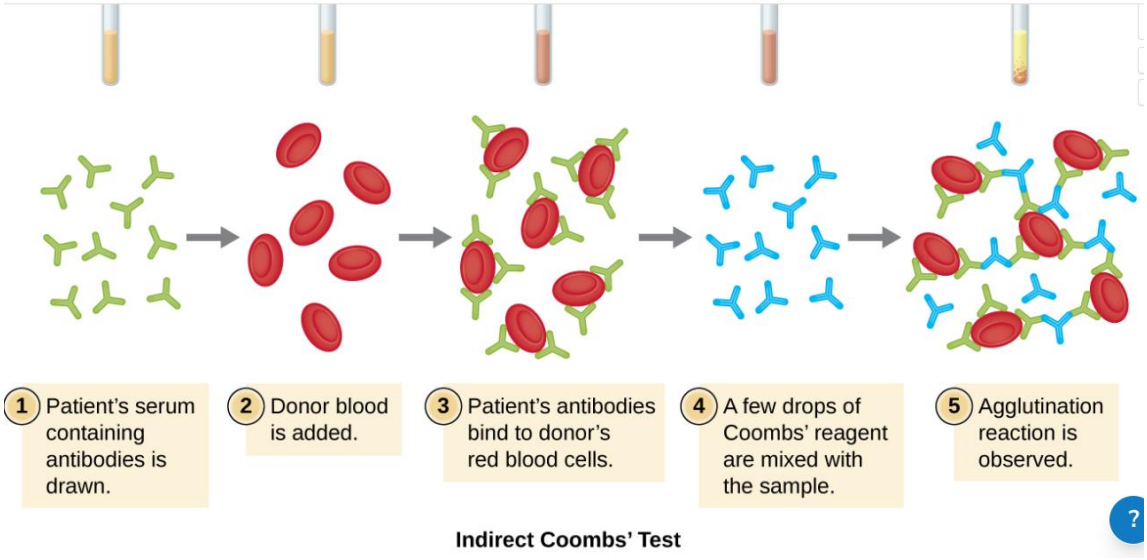


b- Passive or Indirect Hemagglutination

Red blood cells or inert particles can be coated with antigen and used in a passive hemagglutination test. The coupling to red blood cells can be achieved by various methods, the most common being treating the red blood cells with tannic acid, which makes them capable of reacting spontaneously with soluble antigens such as proteins or nucleic acids, or incubating them with the antigens in the presence of glutaraldehyde. The red blood cells here serve as an inert support that does not participate in the antigen-antibody reaction. The presence of agglutinating antibodies will then be detected by the agglutination of the red blood cells to which the antigen has been fixed.

Example of techniques:

Indirect Coombs test: The patient's serum (from which we want to detect the presence of autoantibodies against erythrocytes or anti-Rh antibodies (anti-D) in the mother) is first treated with O blood group red blood cells. The sensitized red blood cells are then reacted with agglutinating antibodies (anti-globulin). A positive reaction is indicated by the agglutination of the sensitized red blood cells.



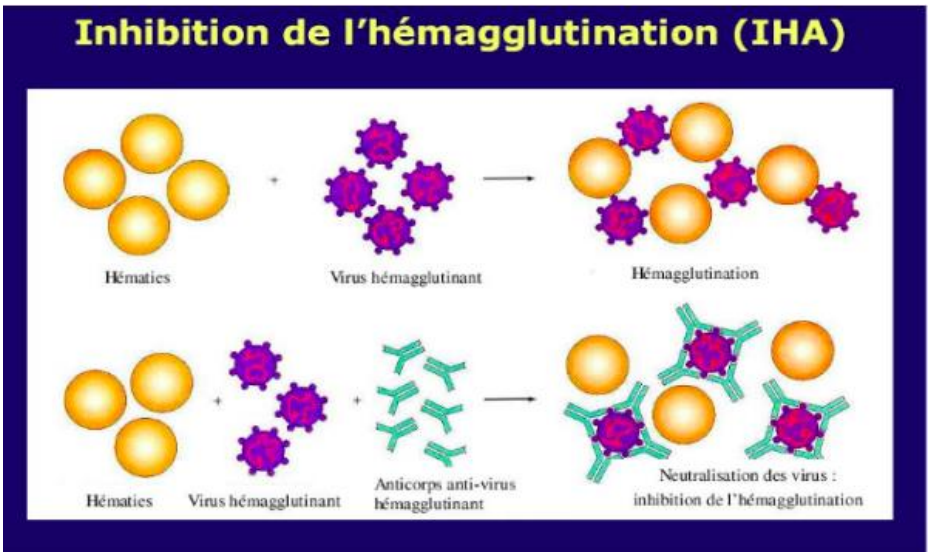
Indirect Coombs' Test

3- Hemagglutination Inhibition

A large number of viruses possess hemagglutinins on their envelope. These allow them to cause agglutination of red blood cells. The hemagglutination inhibition reaction involves a viral antigen, antiviral antibodies directed against this hemagglutinin, and the receptors carried by red blood cells.

In this reaction, the protective antiviral antibodies bind to the virus and prevent it from interacting with the receptors on the red blood cells introduced in the second step of the reaction.

- If the antibodies recognize their specific antigen, the resulting immune complex neutralizes the virus's hemagglutinating ability, leading to a negative hemagglutination result.
- If the antibodies do not match the isolated virus, the immune complex does not form, and the virus remains capable of inducing hemagglutination.



II- Neutralization reaction

1-Neutralization Principle

It is based on the principle that some specific antitoxins or antibodies can reduce or neutralize various biological effects occurring due to different **enzymes, toxins, and viruses.**

It is of two types:

A- **Virus Neutralization Test:** is a type of neutralization test used for virus detection. As the name suggests, it is used for the neutralization of the biological activities of the virus.

The virus contains certain antigenic determinants to which the antibodies initiate the neutralization of the effects of the virus.

B- Toxin Neutralization Test: The toxins produced by microbes are harmful as they can alter different biological functions of our body once injected within. But there are certain antitoxins prepared specifically to reduce the biological effects of those toxins. Utilization of these antitoxins for specific toxin neutralization is the basis of this test.

2- Advantages of Neutralization Test

-It has higher sensitivity.

- It has higher specificity.

- The neutralizing antibodies used in virus neutralization tests can detect the virus along with its various strains.

3-Limitations of Neutralization Test

-The neutralizing antibodies take time to generate in the body after an infection.

- These tests require professionally skilled individuals to perform.

- It requires a great deal of work.

- The materials required for it can be hard to produce and manage.

- These tests require highly protective lab conditions and care as working with the virus can be hazardous.