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Agglutination Reaction

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Learning Objectives :

By the end of this presentation, you will be able to:

1 Define agglutination reaction

2 Differentiate between direct and indirect agglutination tests


3 Applications of agglutination reaction

4 Complement fixation

Agglutination Reaction



When an immune complex is formed by cross-linking cells or particles (Antigens) with specific antibodies, in the presence of electrolytes at a suitable temperature and pH it is called an **agglutination reaction**. Antibodies that produce such reactions are called **agglutinins**.



Agglutination reactions usually form visible aggregates or clumps (Agglutinates**) that can be seen with the unaided eye.**

Agglutination reactions are very sensitive, relatively easy to read, and available in great variety.



Agglutination Tests

Agglutination Tests

```
graph TD; A[Agglutination Tests] --> B[Direct Agglutination Test]; A --> C[Indirect Agglutination Test];
```

**Direct
Agglutination
Test**

**Indirect
Agglutination
Test**

The slide features decorative geometric shapes. In the top-left corner, there are several overlapping, semi-transparent polygons in shades of blue, green, and purple. In the bottom-right corner, there are several overlapping, semi-transparent polygons in shades of light gray.

Direct Agglutination Tests



Direct agglutination tests detect antibodies against relatively large cellular antigens, such as those in red blood cells, bacteria and fungi.

At one time they were carried out in a series of test tube, but now they are usually done in a plastic *microtiter plates*.

These tests are used, for example, to test for brucellosis and Salmonella.

The slide features decorative geometric shapes. In the top-left corner, there are several overlapping, semi-transparent polygons in shades of light blue, teal, light green, and light purple. In the bottom-right corner, there are several overlapping, semi-transparent polygons in shades of light grey and white.

Indirect (Passive) Agglutination Testes

Carrier particles

Soluble antigen

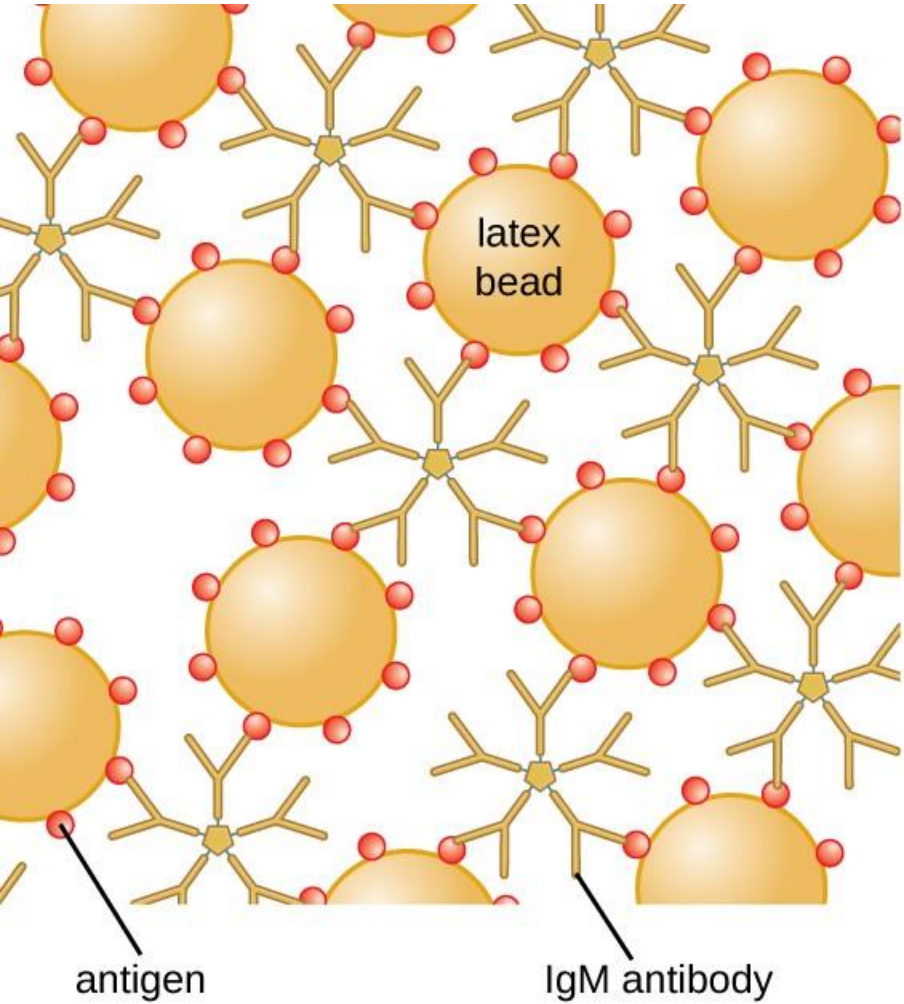


**Fixed on
them**

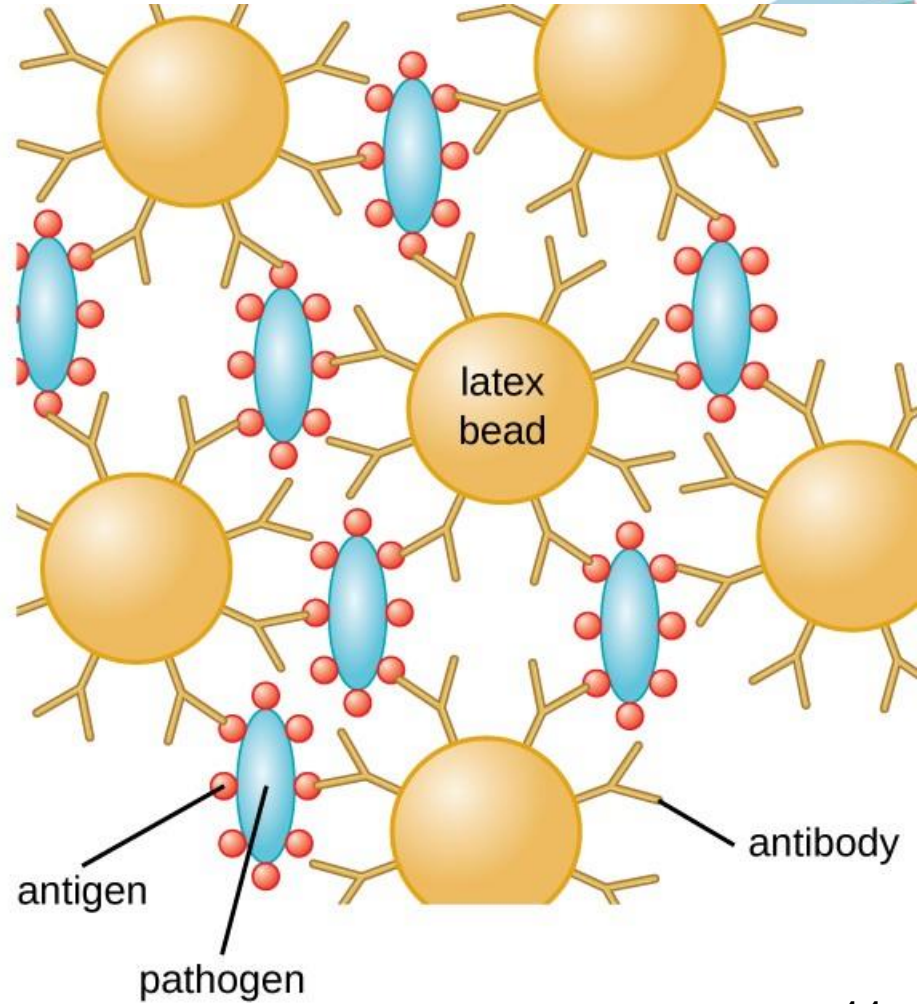
**Specific
antibodies**



Agglutination



(a) positive agglutination test for antibodies




(b) positive agglutination test for antigens



The commonly used carrier particles are latex particles, polystyrene or bentonite.

This approach is especially common in tests for the streptococci that cause sore throats. A diagnosis can be completed in 10 minutes.

The slide features decorative geometric shapes in the corners. The top-left corner contains several overlapping triangles in shades of blue, green, and purple. The bottom-right corner contains several overlapping triangles in shades of grey.

Applications of Agglutination Reaction



01

Slide Agglutination

1. **Uniform suspension of particulate antigen.**
2. **Distinct clumping within 60 seconds is a positive result.**

USES:

For the identification of unknown bacterial cultures.

Also the method for blood grouping.



Slide Agglutination Test



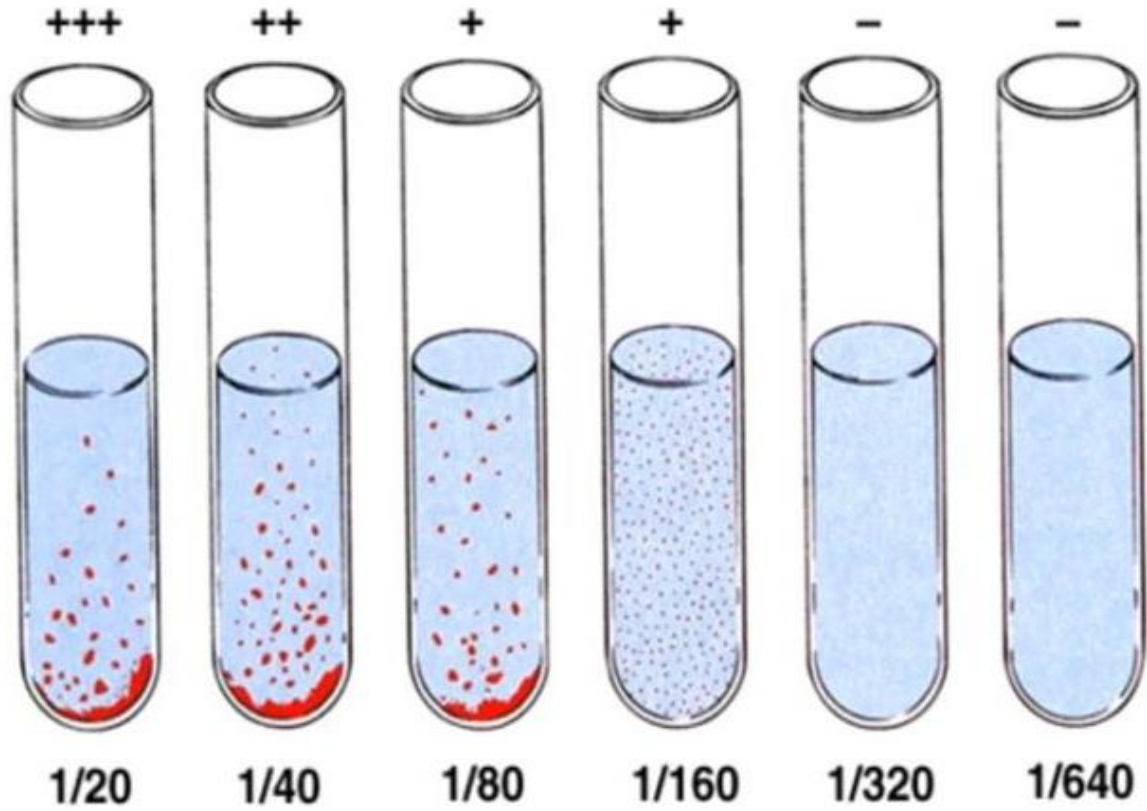
02

Tube Agglutination


1. Is a quantitative method.
2. Particulate antigen and equal volume of serial dilution.
3. Agglutination titer.

USES:

For diagnosis of typhoid, brucellosis and typhus fever.



Titer = 160



For diagnostic purposes, a rise in titer is significant. If it can be demonstrated that the person's blood had no antibody titer before the illness but has a significant titer while the disease is progressing, this change, called **Seroconversion.**

This situation is frequently encountered with HIV infections.

03

AntiGlobulin (Coombs') Test

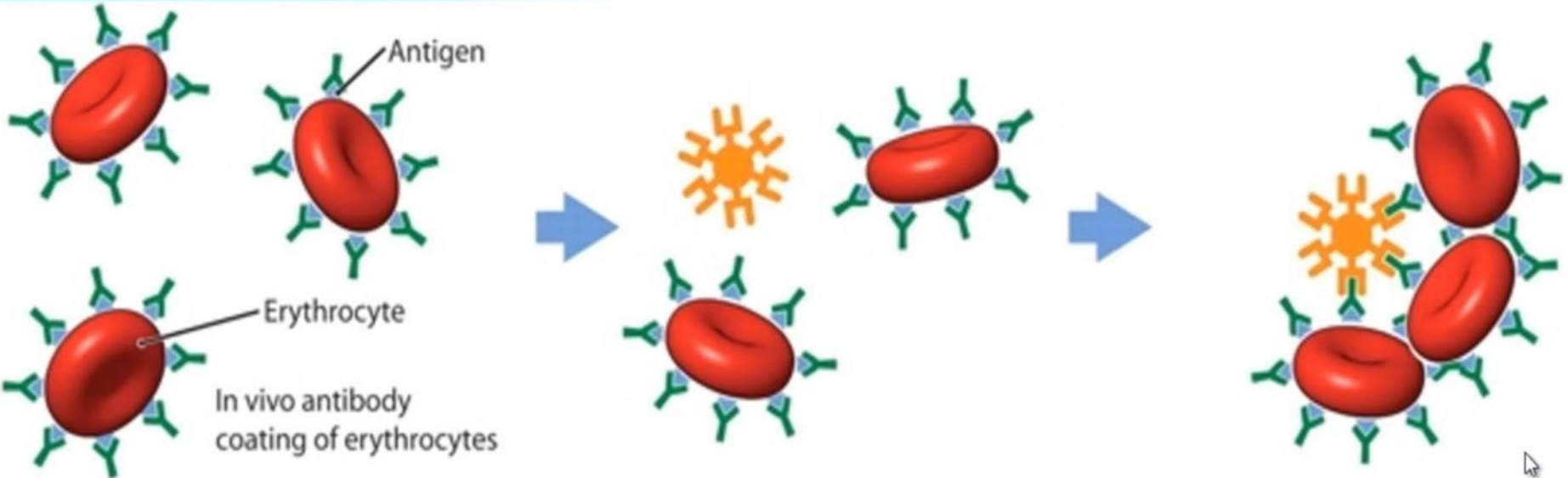
Types of Coombs' Test :

Coombs test is of two types:

❑ Direct Coombs' test:

The sensitization of the erythrocytes with incomplete antibodies takes place in *vivo* as in case of hemolytic disease of the newborn due to Rh incompatibility.

Direct Antiglobulin Test



➔ Anti-IgG AHG reagent added after erythrocytes are washed

➔ AHG reagent causes IgG-coated erythrocytes to agglutinate



□ Indirect Coombs' test:

Sensitization of RBCs with incomplete antibodies is performed in *vitro*.

USES:

Erthroblastosis fetalis diagnosis.

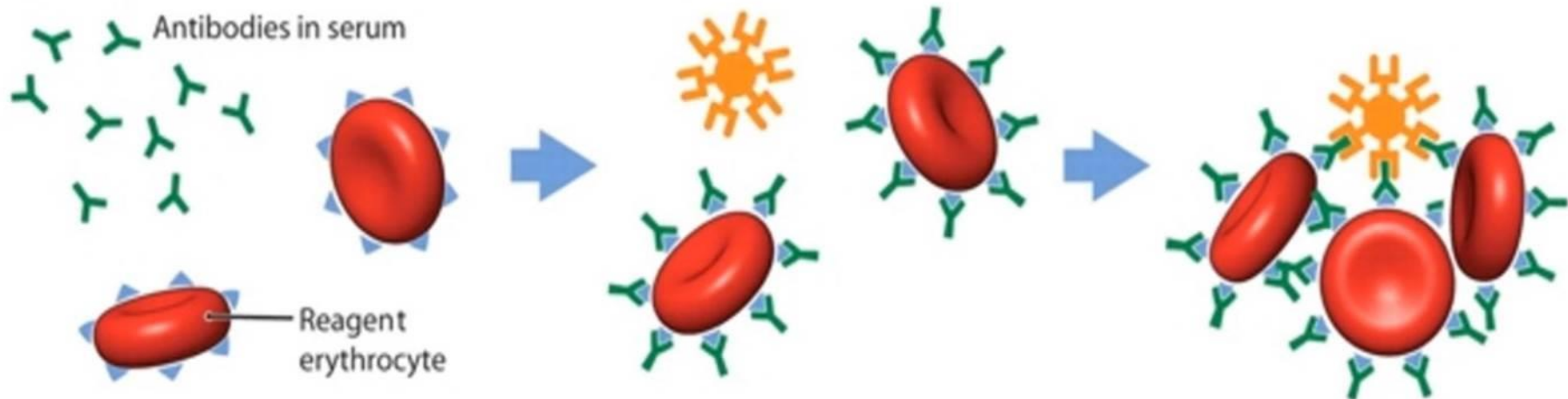
Detection of Rh antibody in :

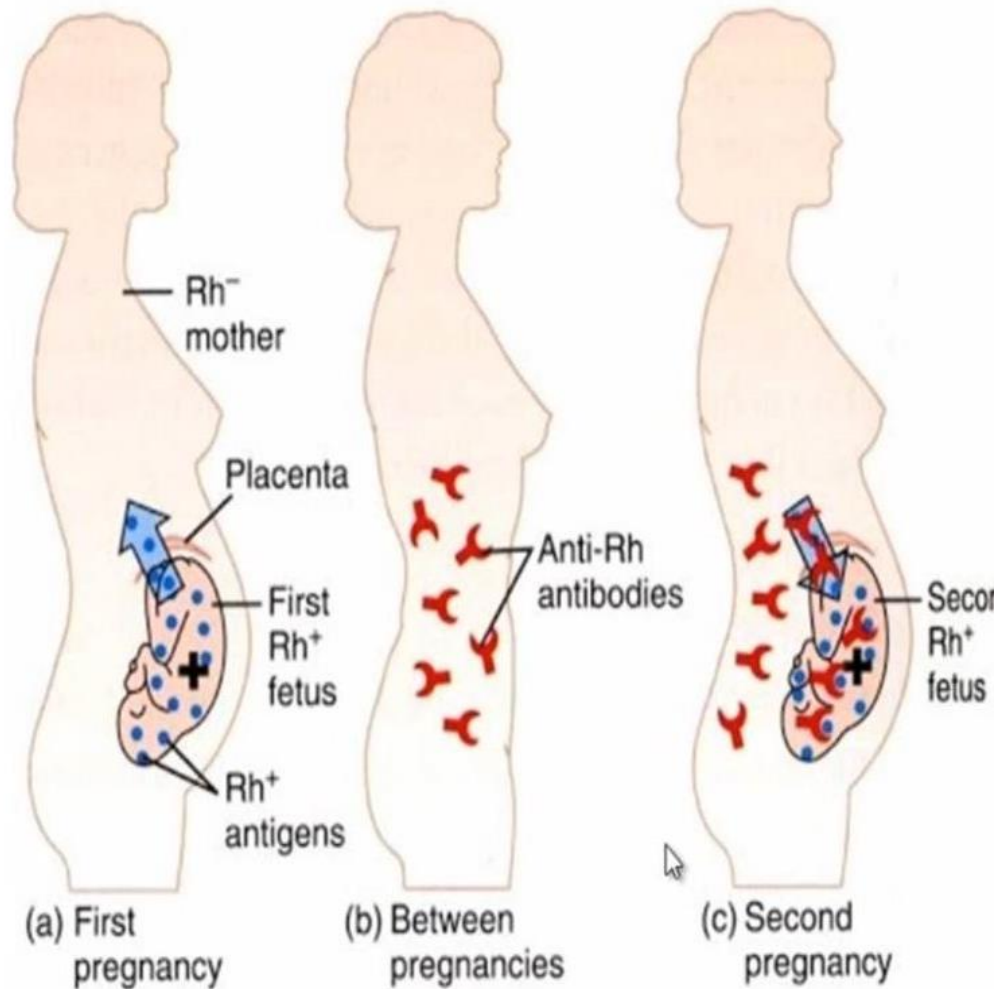
- **Mother's serum.**

➔ Anti-IgG AHG reagent added
after erythrocytes are washed

➔ AHG reagent causes IgG-coated
erythrocytes to agglutinate

Indirect Antiglobulin Test



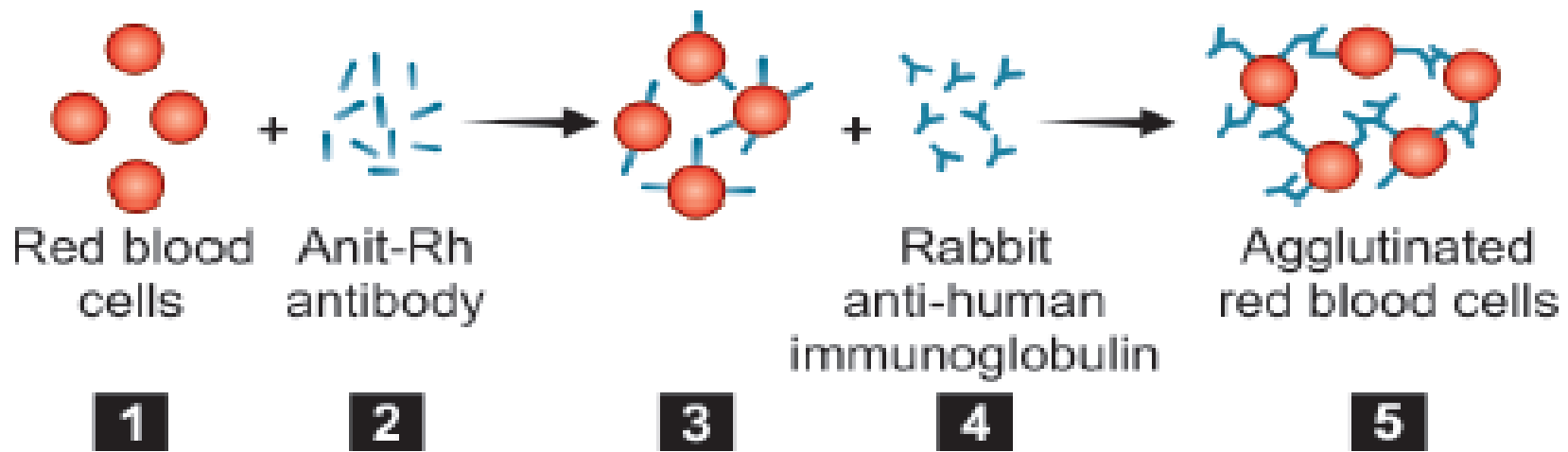




04

Passive Agglutination





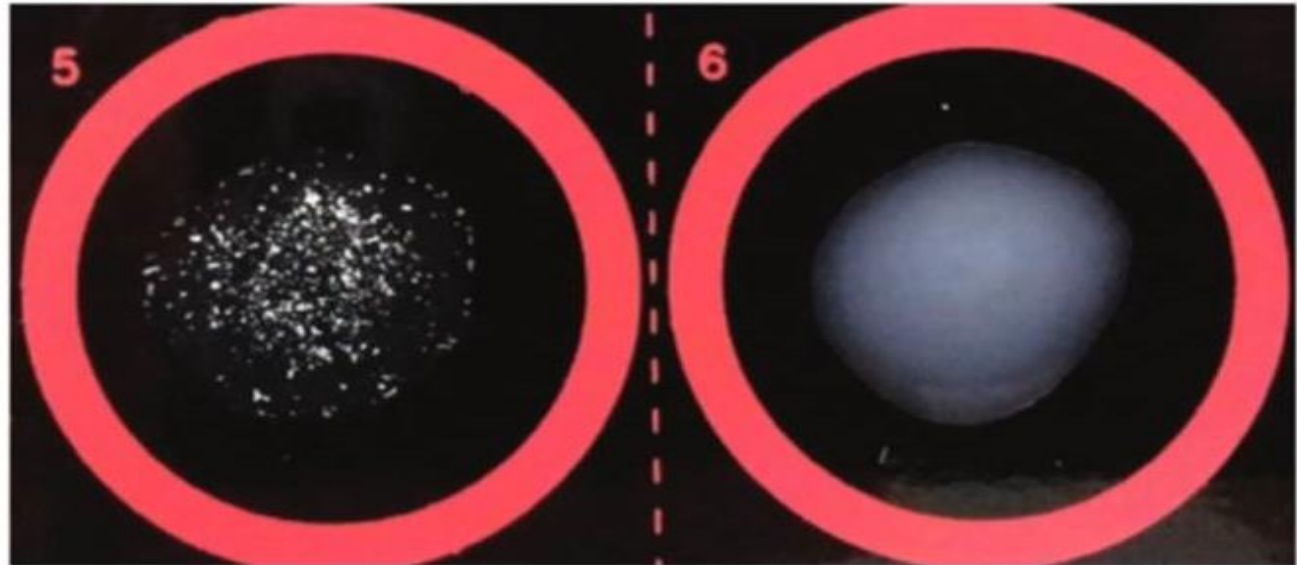
Rh-positive erythrocyte:

1. are mixed with incomplete antibody.
2. antibodies coats the cells.
3. being incomplete, cannot produce agglutination. on addition of antiglobulin serum.
4. which is complete antibody to immunoglobulin, agglutination takes places.

Examples of passive agglutination

1 . Latex agglutination test:

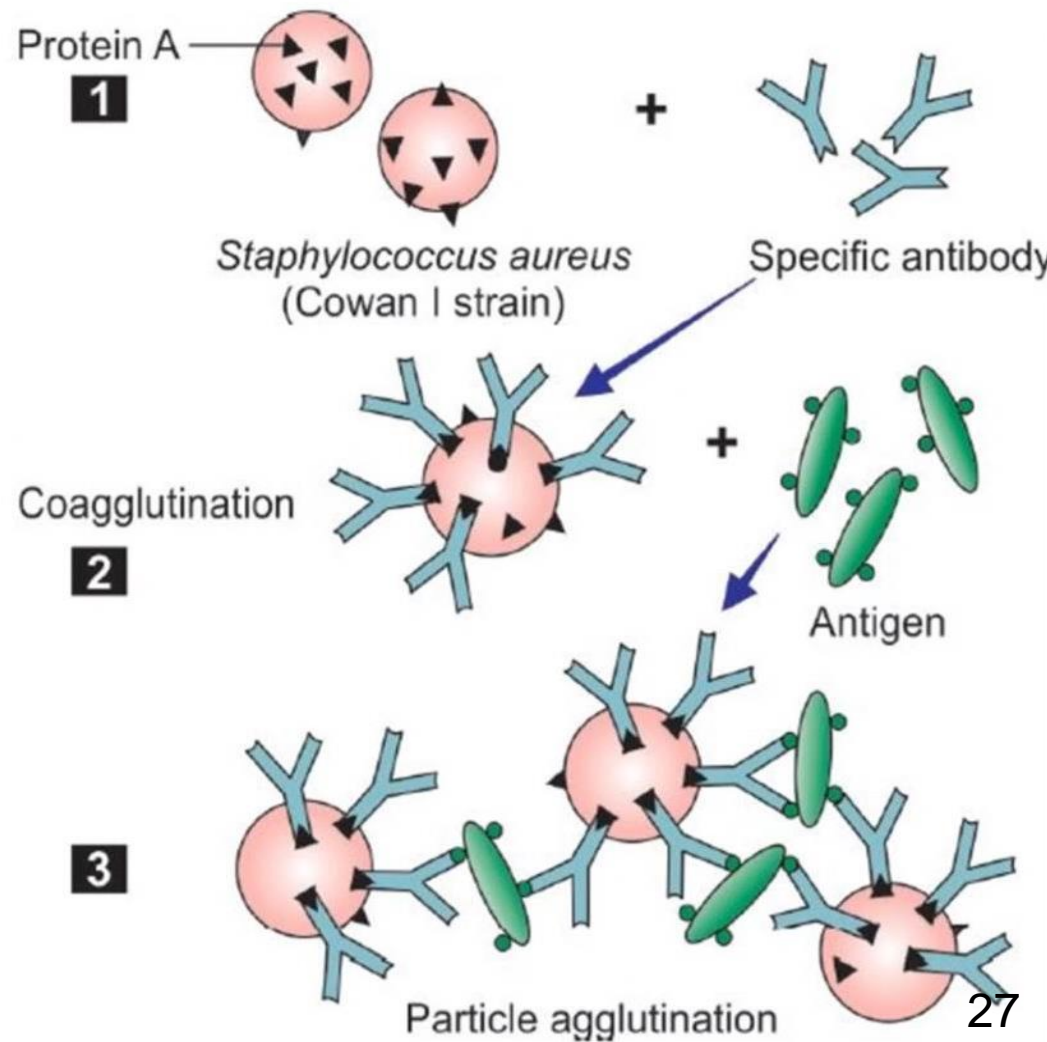
For Rubella test



Positive LAT

Negative LAT

2 . Co-Agglutination test:



Complement fixation



- **Complement usually does not bind to free antibody or free antigen.**
- **Ag-Ab complex fix complement :
complement only bind to bound antibody
(Ab attached to Ag) at Fc portion.**
- **Compliment kills the antigen brining cell to
which antibody is attached to.**

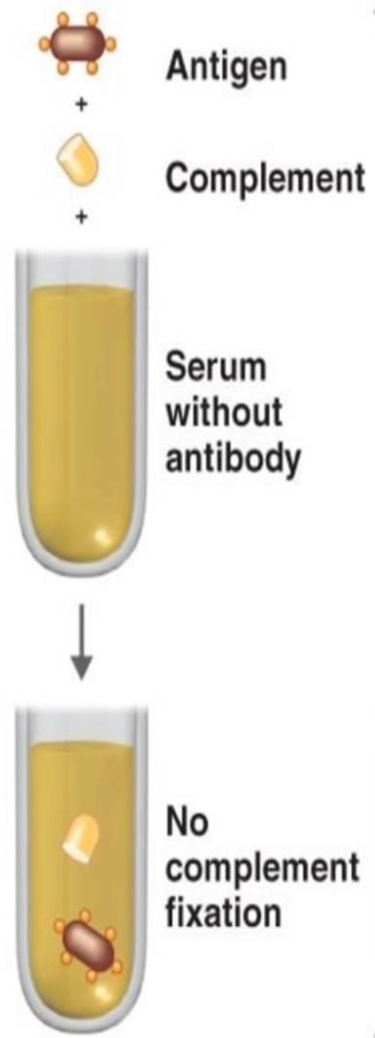
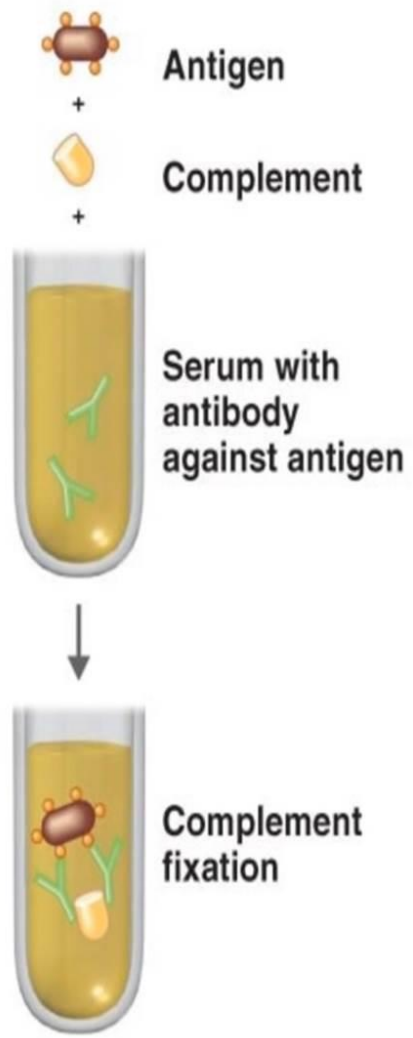
Complement fixation



- CFT is used to detect antibodies.

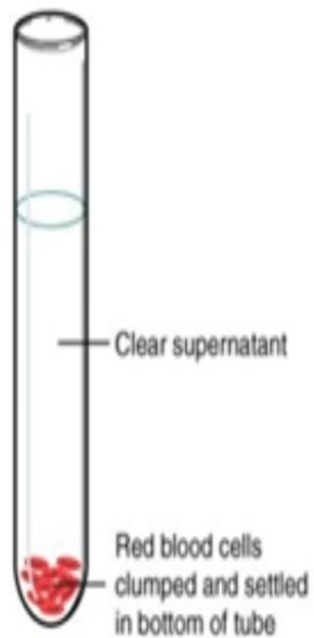
CFT requires :

- Antigen, Antibody, Complement (Guinea pig).
- sheep erythrocytes and amboceptor (rabbit antibody to sheep red cells).



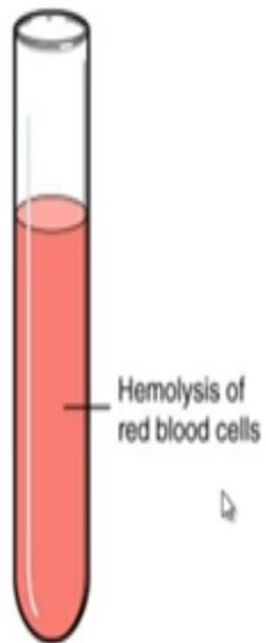
Complement-fixation stage

Result: No Hemolysis



Positive CFT

Result: Hemolysis



Negative CFT

Summary

- **We defined agglutination which is an immune complex.**
- **Agglutination tests are classified as either direct or indirect.**
- **Applications of agglutination reaction:**
 - **Slide Agglutination Test.**
 - **Tube Agglutination Test.**
 - **Antiglobulin (Coobs) Test.**

Summary

- **Passive Agglutination:**
 - Latex agglutination test.
 - Co-Agglutination test.
- **Compliment kills the antigen brining cell to which antibody is attached to.**

Reference



Essentials of Medical Microbiology 2016

Tortora-Funke-Case Microbiology an introduction 6th ed.

Prescott Harley Klein Microbiology 6th ed.



Thank you