Antigen-Antibody Reactions

Agglutination

Agglutination

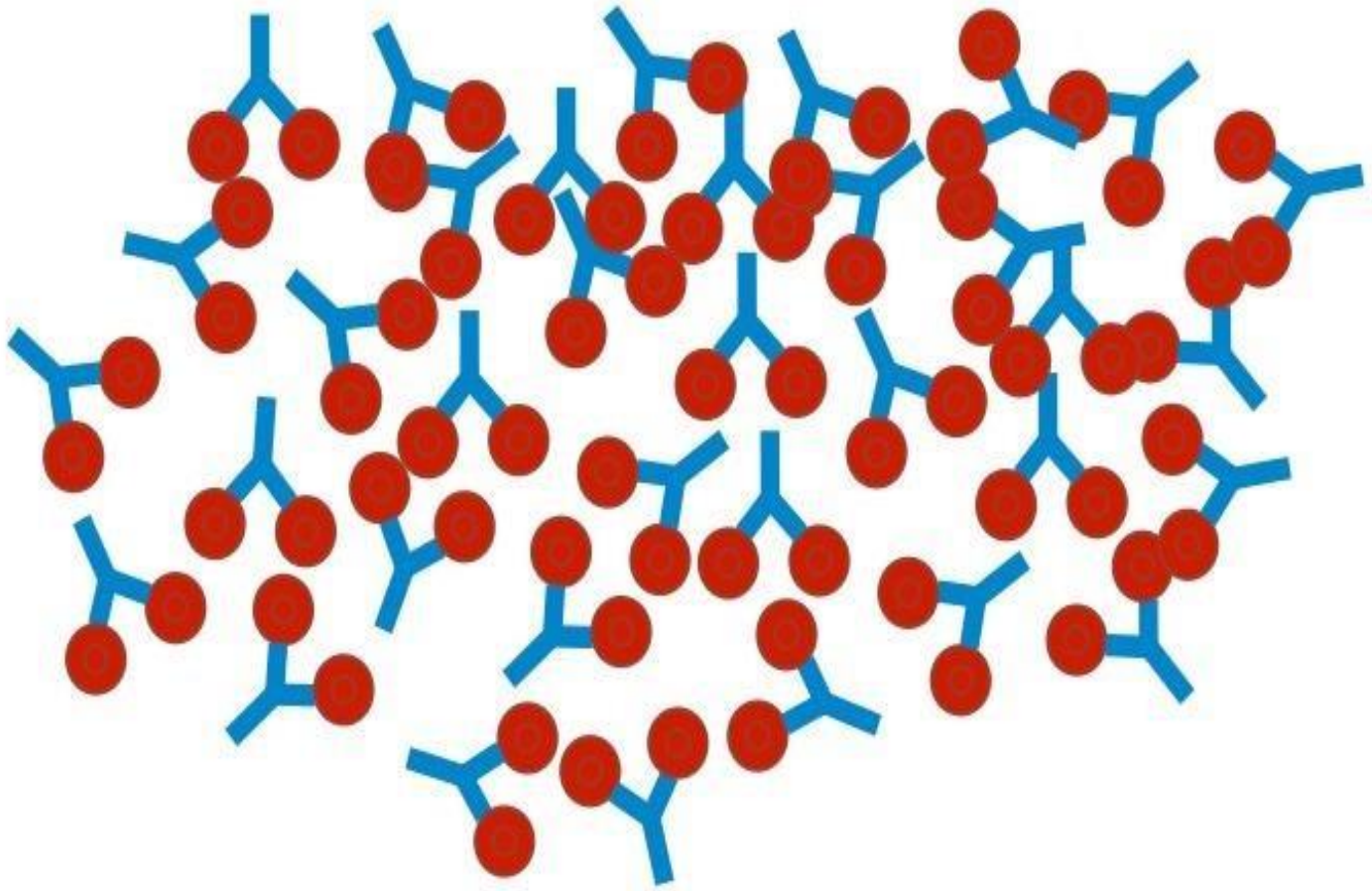
- When a particulate antigen* combines with its specific antibody at a suitable temperature and pH, and this interaction between the antibody (Ab) and particulate antigen results in visible Ag clumping.

- particulate antigen (Ag)+ specific Ab $\xrightarrow[\text{temperature and pH}]{\text{Suitable}}$ visible Ag clumping (Agglutination)

- Optimal reaction occurs when Ags and Abs are in equivalent proportions** (Zone of equivalence)

*cells that carry antigenic molecules (multiple antigenic sites that can induce an immune response.) on their surface for example whole bacterial cells and fungal cells.

Agglutination: The action of an antibody when it cross-links multiple antigens producing clumps of antigens

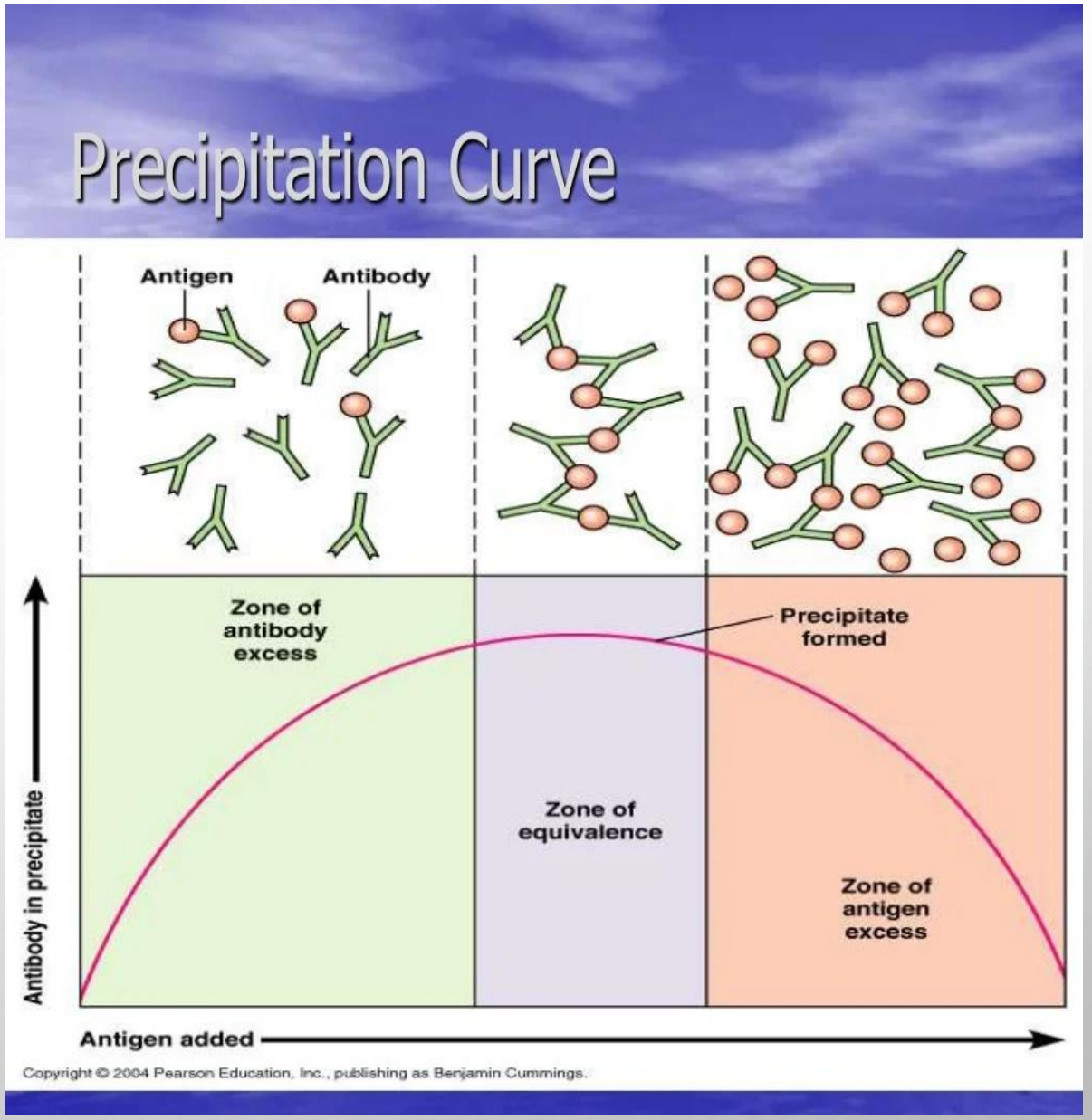


LATTICE THEORY

Zone of antibody excess (Prozone)
precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant

Zone of equivalence
Maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant;

Zone of antigen excess (Postzone)
Precipitation is inhibited & Ag. not bound to Ab. can be detected in the supernatant



Agglutination tests

1-Active (Direct) agglutination

1. Slide agglutination test
2. Tube agglutination test
3. Heterophile agglutination test
4. Antiglobulin (Coomb's) test

a. Direct Coomb's test

b. Indirect Coomb's test

2-Passive (Indirect) agglutination

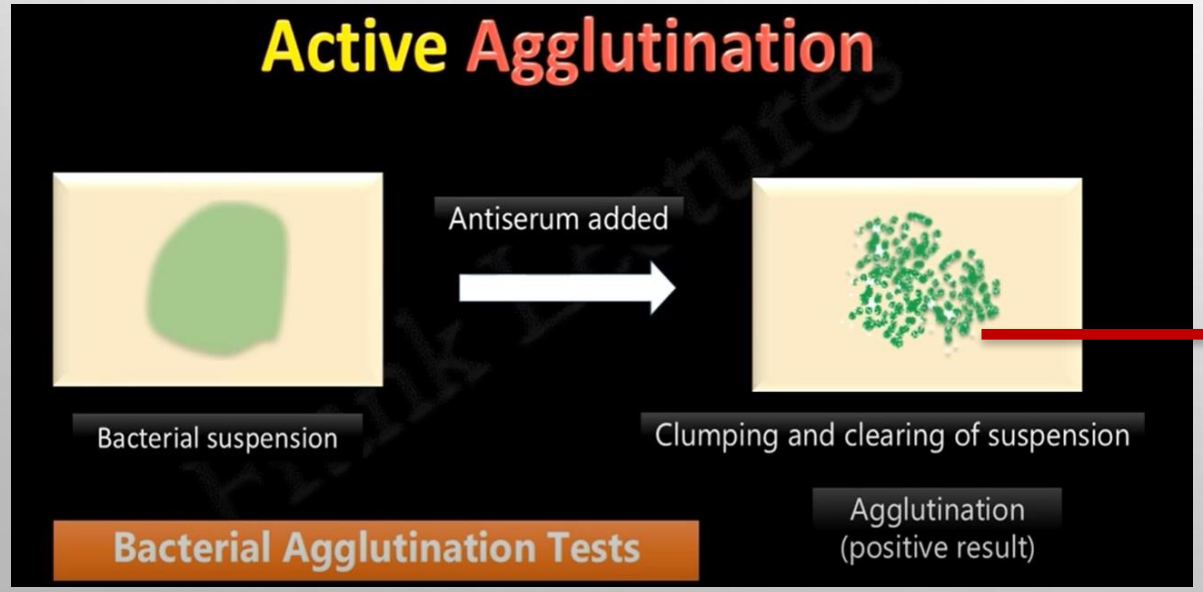
Ex; Latex agglutination

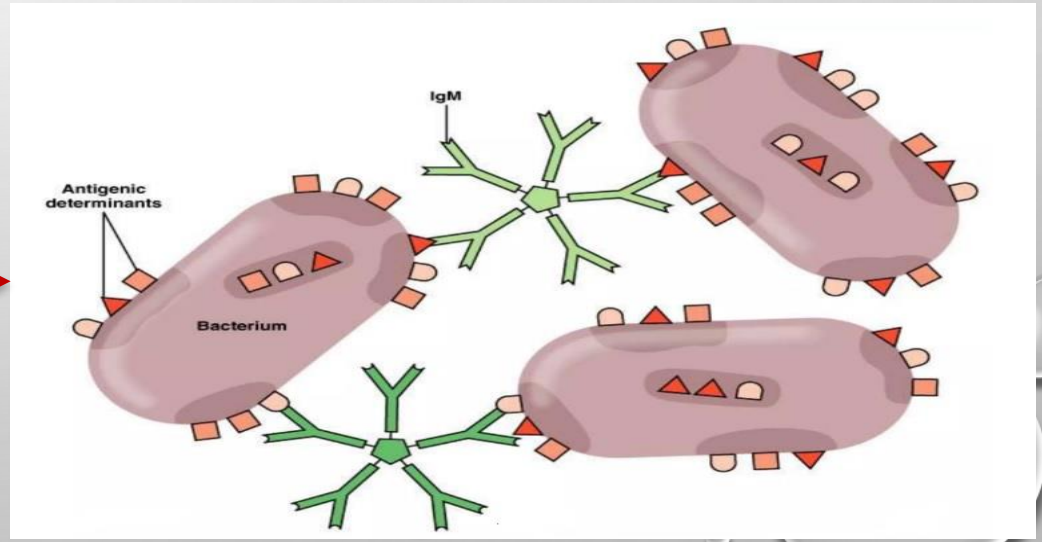
Types of Agglutination reactions

1-Active (Direct) agglutination

- **Epitopes of interest are found naturally** on the test particle. Ex: Bacterial cells etc. In this case **Abs can bind directly to these antigens** and agglutinate them.

- Direct agglutination of particulate antigen with specific antibody occurs.

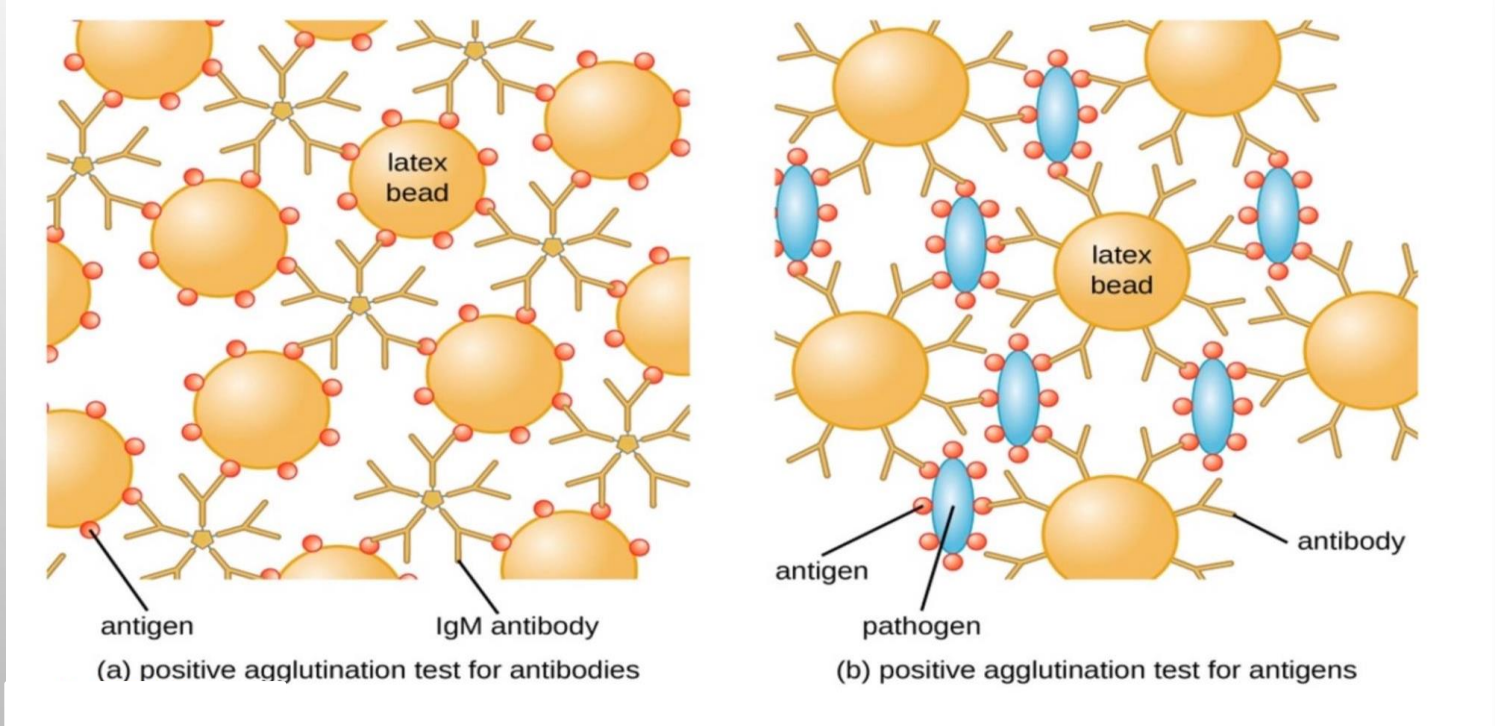




Types of Agglutination reactions

2-Passive (Indirect) agglutination

- The epitope of interest **does not occur naturally** on the cells or particles to be agglutinated In this case the antibody or antigen is attached to certain **inert carrier** (ex: **Latex beads**)



Passive Agglutination Test

**Reverse Passive Agglutination Test

****When the antibody instead of antigens is adsorbed on the carrier particle for detection of antigens**

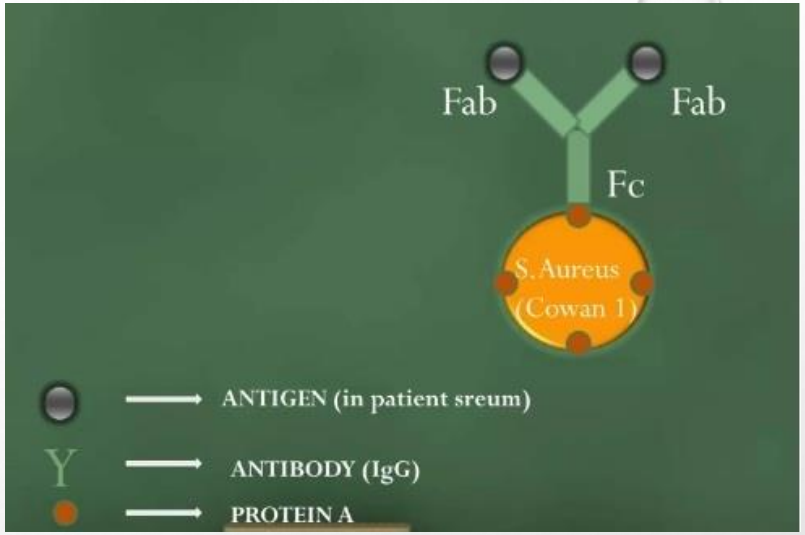
- The particles (in this case, latex) do not themselves play a part in the reaction and they are therefore PASSIVE

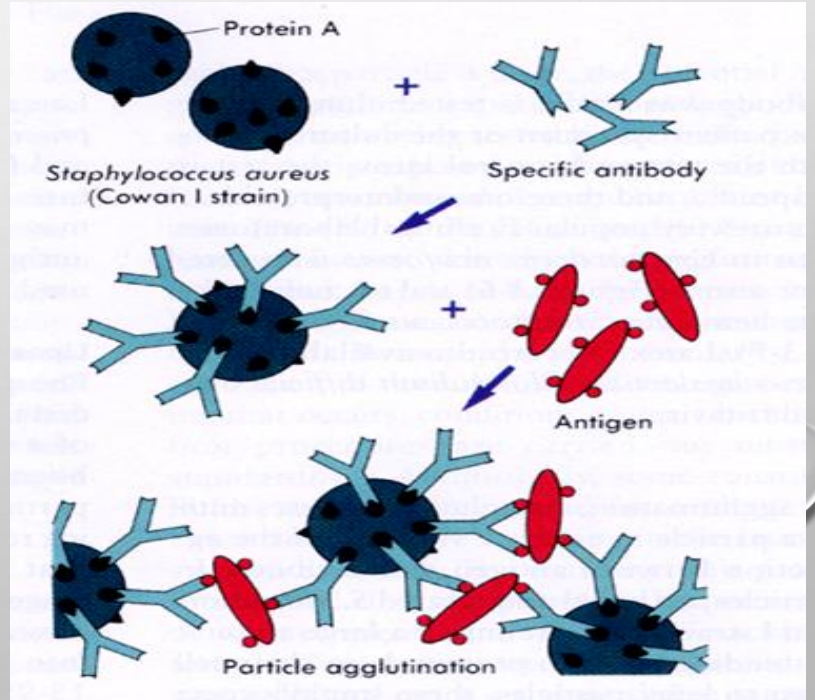
(146) Latex Agglutination - Part 4 - YouTube

(146) Latex Agglutination - Part 7 - YouTube

Co-agglutination

- Similar to latex agglutination
- Ex. ***Staphylococcus aureus*** can be used as carrier molecules (passive agglutination)
- ***S.aureus* has protein 'A' on its cell wall that can bind with Fc region of antibody.**
- Such *S.aureus* coated with specific antibody can be used to detect specific antigen.



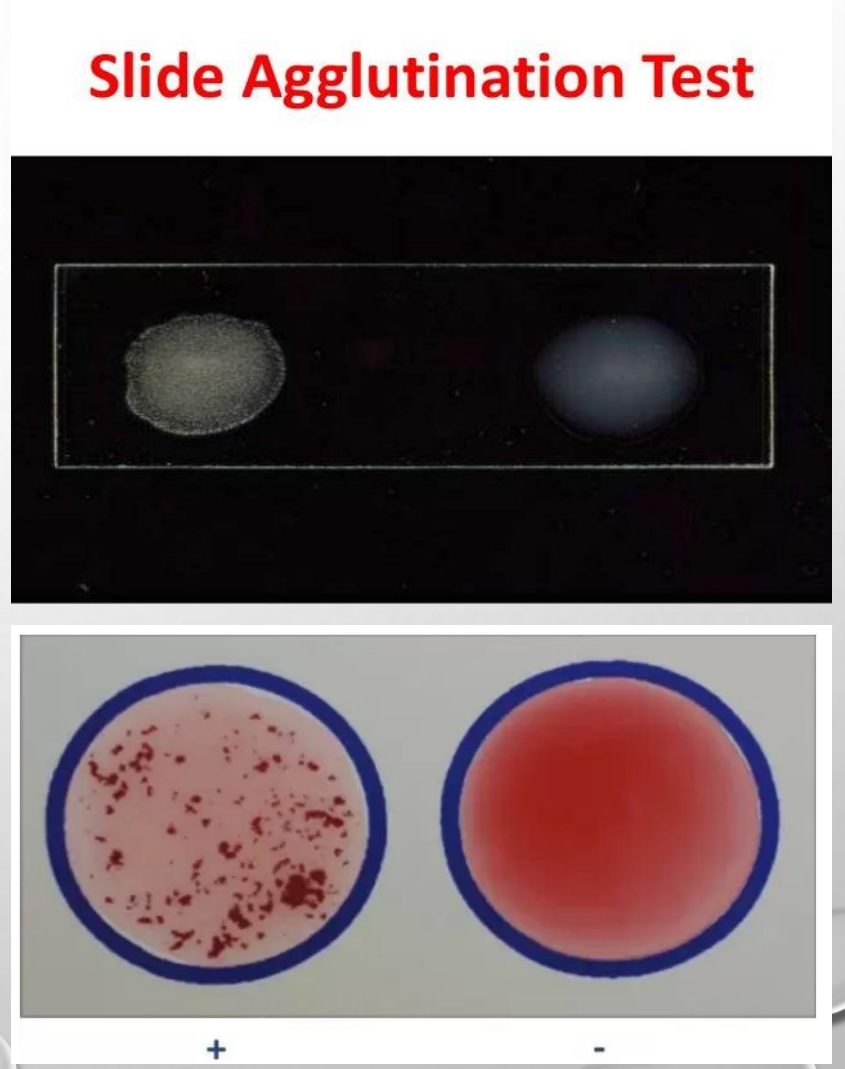


Active (Direct) agglutination

1. Slide agglutination test

- When **appropriate antiserum** is added to uniform suspension of a **particulate antigen**

- **Positive result:** is indicated by the **clumping formation** and **clearing of the background solution** (**Qualitative**).



- **Uses:**

1-Routine procedure for the identification of **bacterial isolates from clinical specimens**.

2-For blood grouping

2-Tube agglutination test (Widal)

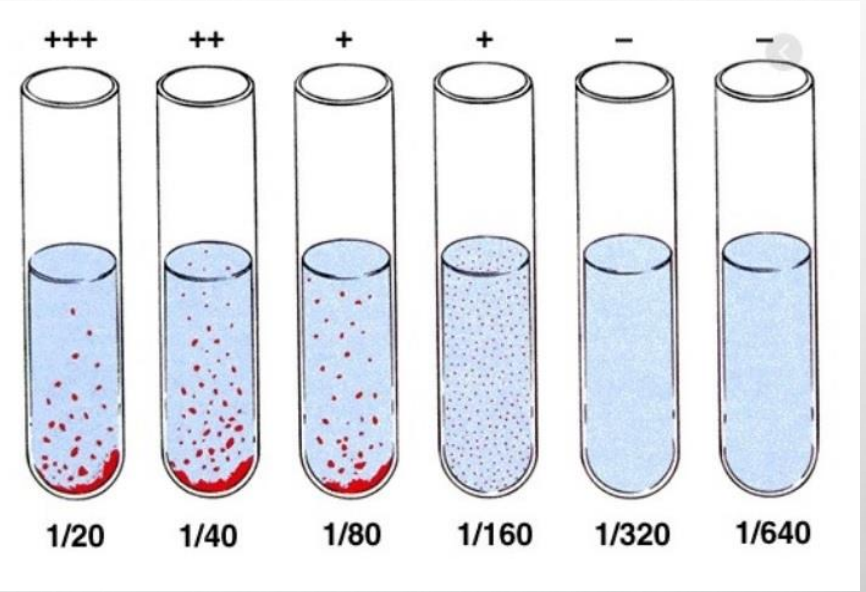
- Standard **quantitative method** for the measurement of **antibodies**

- patient's serum is diluted in a series of tubes and the specific antigens are added to it.

- Antigen and antibody reactions are demonstrated by demonstration of **visible clumps of agglutination**

- **Used for quantitative estimation of antibodies in the serum.**

- for the serological diagnosis of typhoid, and rickettsia fever.



(144) Tube agglutination test - YouTube

3-Antiglobulin (Coomb's) test

- Most people have Rh protein on the red blood cells called the rhesus factor (Ag) these people are (Rh positive)
- small percentage of the global population is missing this protein (Rh negative).

- These two blood groups are **incompatible,** that means that if they mix, **deadly reactions can occur**

- Example: **Erythroblastosis Fetalis (Hemolytic disease of the newborn)**

Erythroblastosis Fetalis

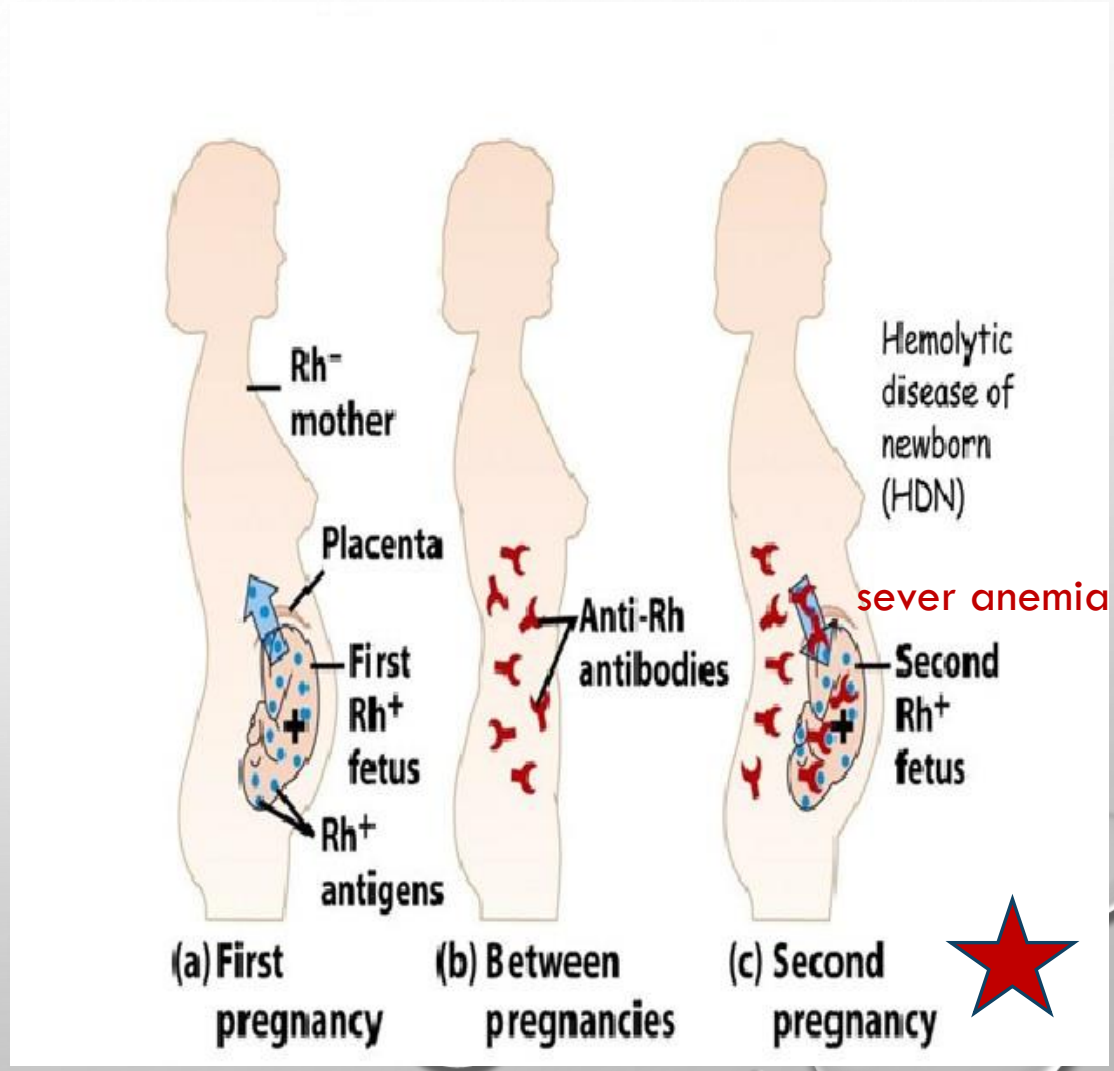
- **Cause: Rh incompatibility**

- **It's dangerous when an Rh-negative woman becomes pregnant with an Rh-positive baby.** (why?/how?)

- If the baby's red blood cells get into the mother's blood stream, the immune system will consider them foreign invaders and create antibodies

- Anti-Rh antibodies can attack the fetus's red blood cells. This can lead to serious health problems, even death, for a fetus or a newborn.

- Coomb'sTest can be used to detect the presence of these Abs



4-Antiglobulin (Coomb's) test

***It determines whether the red blood cells (RBCs) circulating in the bloodstream are covered with antibodies (**These antibodies sometimes destroy red blood cells and cause anemia)

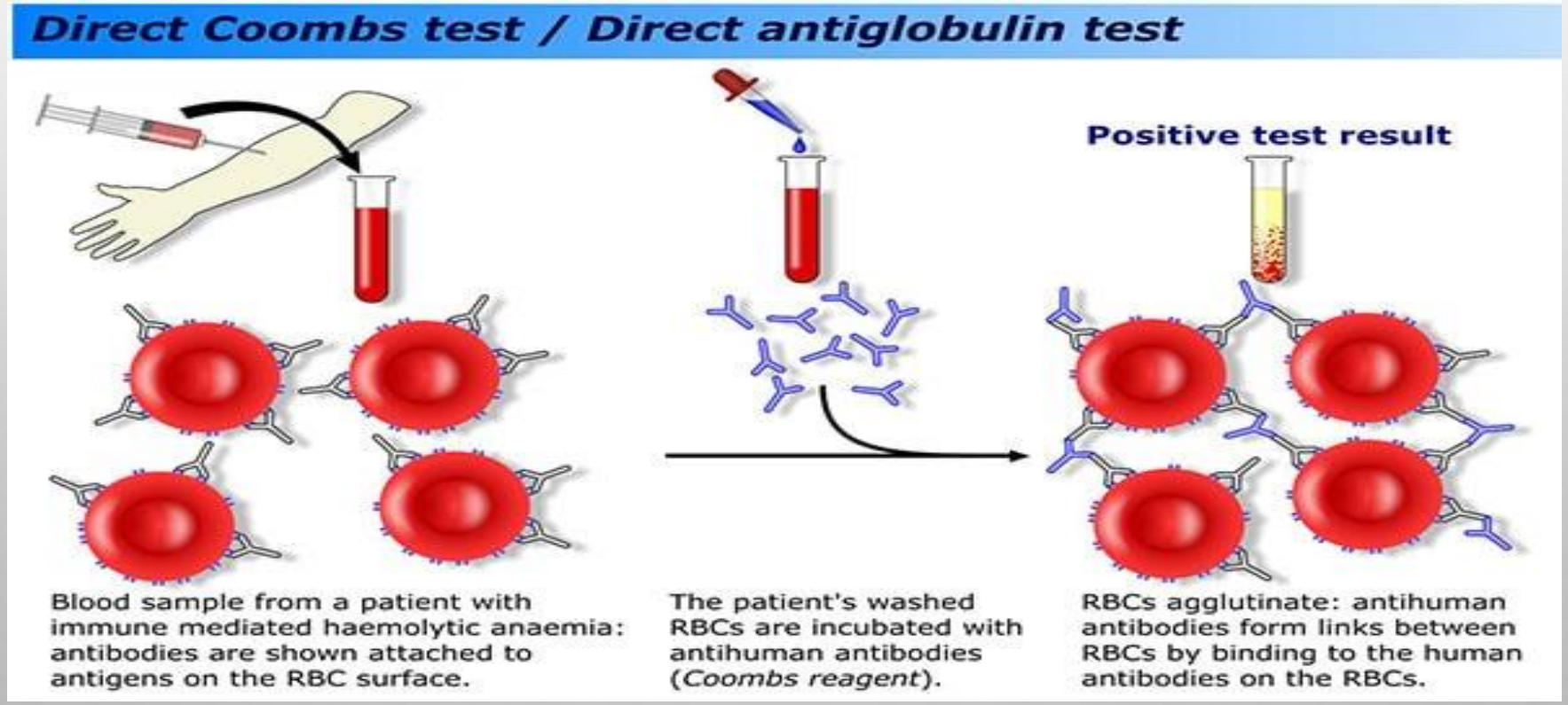
a. **Direct comb's test**
b. **Indirect comb's test**

A- Direct Antiglobuline/ coomb'stest (DAT)

These antibodies that **bind to RBC but do not cause agglutination** are known as **incomplete antibodies (**non-agglutinating antibodies)

In order to detect the presence of non-agglutinating antibodies on red blood cells, we can use

a second antibody directed against the immunoglobulin (antibody) coating the red cells.
This anti- immunoglobulin can now cross link the red blood cells and result in agglutination.



B- Indirect Antiglobulin/ coomb'stest (IAT)

To detect any **free-flowing antibodies against certain red blood cells**. It is most often done **to determine if you may have a reaction to a blood transfusion.**

This test is done by **incubating the red blood cells** with the **serum sample**, washing out any unbound antibodies and then adding **a second anti-immunoglobulin reagent** to cross link the cells.