

Serological tests 1
(Antigen antibody interactions)
Lab 2

Samer Alqaraleh,
PhD. Nanobiotechnology
Faculty of Medicine, Mutah university
Immunology, 2nd year students

What is serology

Serology: is the scientific study of serum and other fluids in *vitro*. In practical, the term usually refers to the diagnostic identification of antibodies or antigens in the serum.

- Infection
- Blood typing
- Autoimmune diseases
- Immune deficiency as X-linked agammaglobulinemia

Antibody molecules combine **reversibly** with antigens to form immune complexes.



Characteristics of this reaction

- Specific
- Optimum temp. 37-56c.

Classification of antigen-antibody interactions:

1. Primary serological tests: (Marker techniques) e.g.

– **Enzyme linked immuno sorben assay (ELISA)**

– Immuno flurescent antibody technique (IFAT)

– Radio immunoassay (RIA)

2. Secondary serological tests: e.g.

A– **Agglutination** tests

B– **Precipitation** tests

C– **Complement fixation tests (CFT)**

D– Serum neutralization tests (SNT)

E– Toxin-antitoxin test

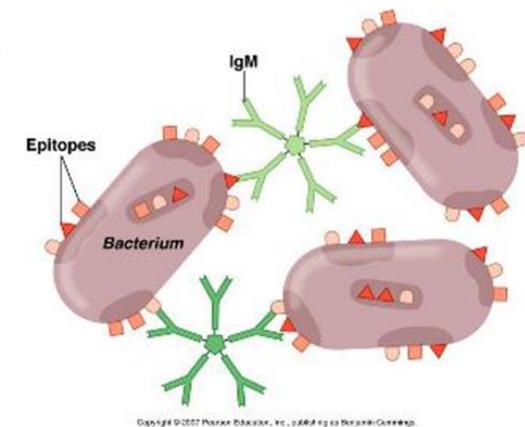
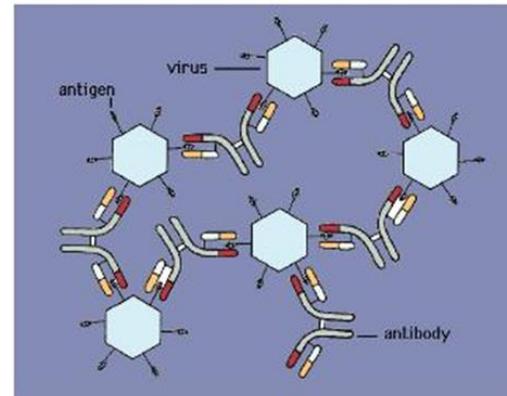
Secondary serological tests:

- In Ag-Ab binding; **Precipitation**, Precipitation reactions are based on the interaction of antibodies and antigens. They are based on **two soluble antigen and antibody** that come together to make one insoluble product, the precipitate which appear as line between 2 solutions.
- In Ag-Ab binding, **Agglutination**; Agglutination is the visible expression of the aggregation of antigens and antibodies. Agglutination reactions apply to cell bound antigens (on RBC or artificially fixed on particles-**particulate**) bind to antibody. The endpoint of the test is the observation of clumps resulting from that antigen-antibody complex formation.

- The general term **agglutinin** is used to describe antibodies that agglutinate particulate antigens (**agglutinogen**). When the antigen is an erythrocyte the term **heamagglutination** is used.

AGGLUTINATION

- ❖ Abs can bind and cross-link cells or particles □ aggregate formation
- ❖ Entrap microbial invaders
- ❖ IgM & IgA are the most suitable (*IgG in sufficient amounts can agglutinate cells*)



Type of agglutination reactions

1. Direct agglutination.
2. Indirect or passive agglutination.
3. Reverse Passive agglutination.
4. Agglutination Inhibition.
5. Coagglutination

These reactions take part in two stages,

1. **Sensitization**, the antibody binds to the red cell or sensitizes it.
2. **Agglutination**. In the second stage, the sensitized red cells agglutinate.

1. Direct Agglutination

(Active)

Direct agglutination test is divided into two classes, such as;

A. Slide Agglutination:

In this method, blood samples are mixed with Anti-A, Anti-B, and Anti-D antibody on a slide to perform the agglutination.

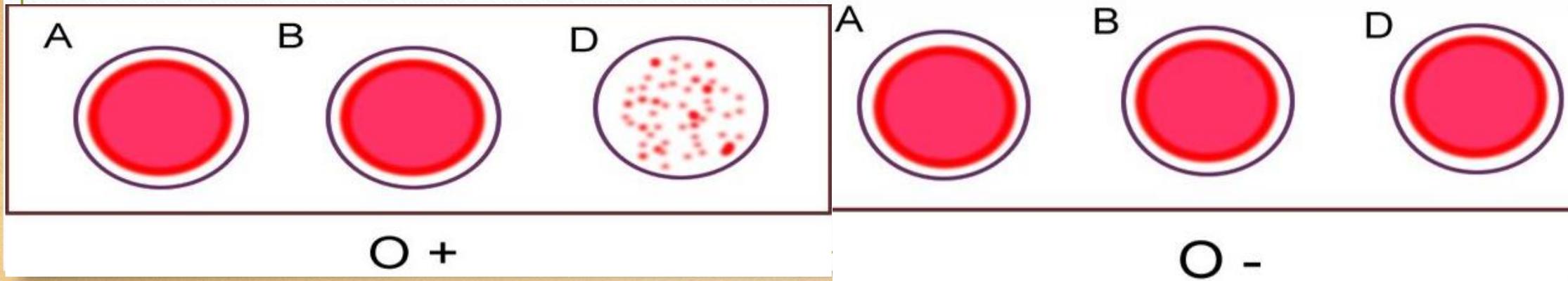
Patient	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Pos.	Neg.	Titer
1	●	●	●	●	●	●	○	○	○	○	●	○	64
2	●	●	●	○	○	○	○	○	○	○	●	○	8
3	●	●	●	●	●	●	●	●	●	○	●	○	512
4	○	○	○	○	○	○	○	○	○	○	●	○	<2
5	●	●	●	●	●	○	○	○	○	○	●	○	32
6	○	○	●	●	●	●	●	○	○	○	●	○	128
7	●	●	●	●	●	○	○	○	○	○	●	○	32
8	●	●	○	○	○	○	○	○	○	○	●	○	4

Application:

- This method is used for the identification of bacteria from clinical specimens.
- used for blood grouping

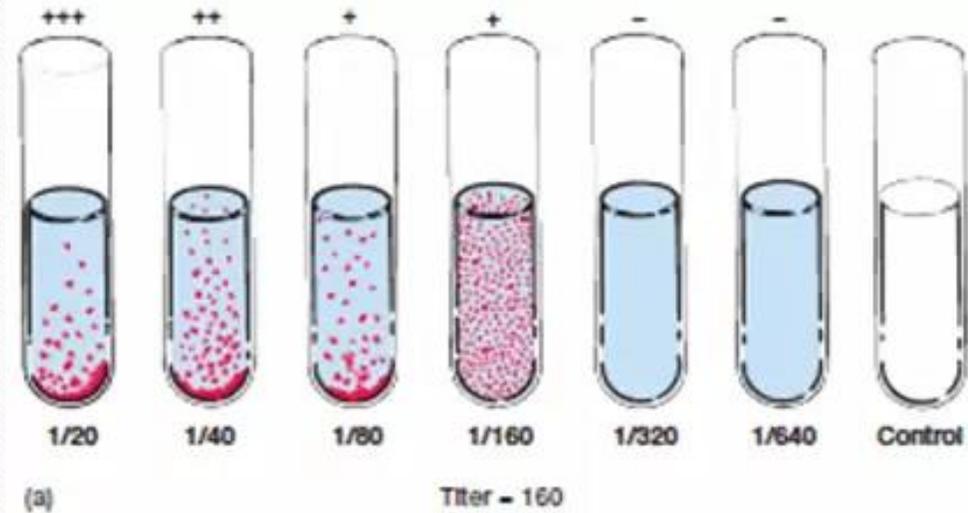
Example:

1. If antigen on red blood cells, then it is called hemagglutination.
 - Uses of heamagglutination are Blood grouping & Cross matching, Antisera of the IgM type can be used to in blood grouping.
 - Smooth suspension of blood on 3 slides + drop of antibody (anti-A, anti B and anti RH on each slide.
 - Clumping of blood means it has that antibody specific antigen
2. Used in identification and typing of micro-organisms as pneumococci



B. Tube Agglutination:

- In this test, serial dilutions are made of an antibody sample (patient serum) and then a constant amount of antigen is added.
- Then the last dilution that gives agglutination is determined and called the **titer**. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.



Application:

- Used for brucellosis test.
- Used in Widal test for diagnosis of for the diagnosis of Typhoid fever.
- The antigens used in this procedure include Salmonella O (somatic) and H (flagellar) antigens.

Titer

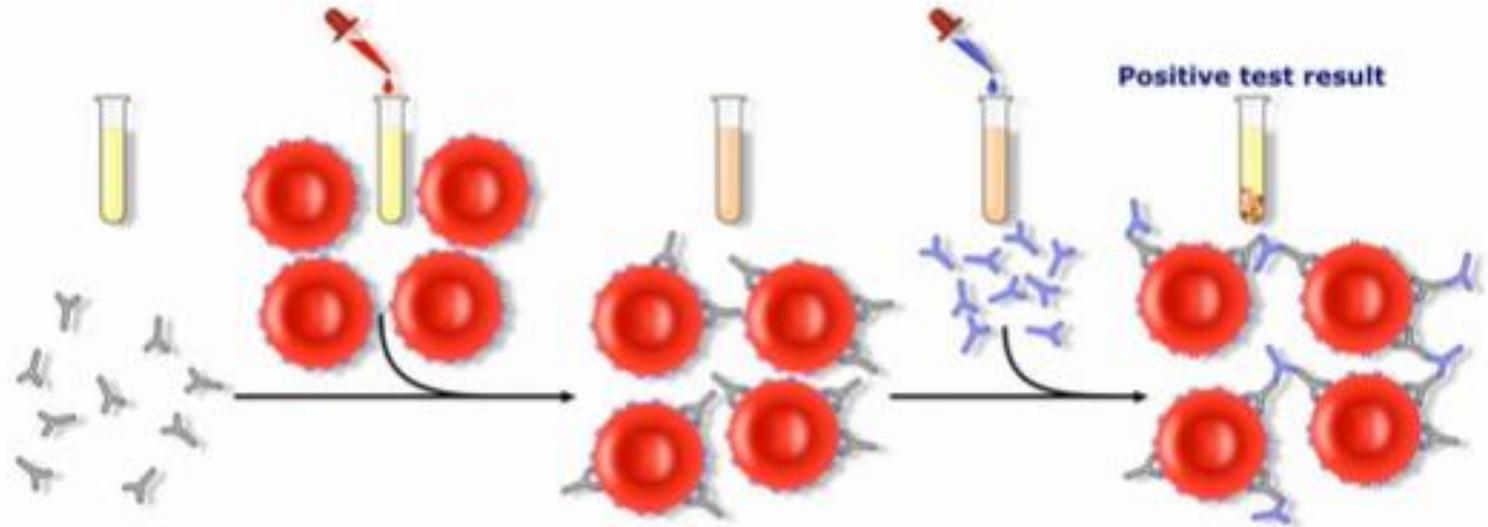
The level of antibody in serum is expressed as the highest dilution of antibodies that gives a positive reaction with antigen. It can be diagnostic or prognostic.

2. Indirect Agglutination or passive agglutination

- When a soluble antigen used in an agglutination reaction it is often coated on a carrier particle, and agglutination takes place on the surface of the carrier molecule.
- In indirect agglutination test RBCs, latex or bentonite, etc used as carrier molecules.
- Used in *Treponema pallidum* (TPHA). Cause syphilis

Indirect combs' test

- Also called Co agglutination
- Anti- Anti-human globulin bind with antibody from Fc



- ▶ For detection of incomplete Ab in the serum of the mother.

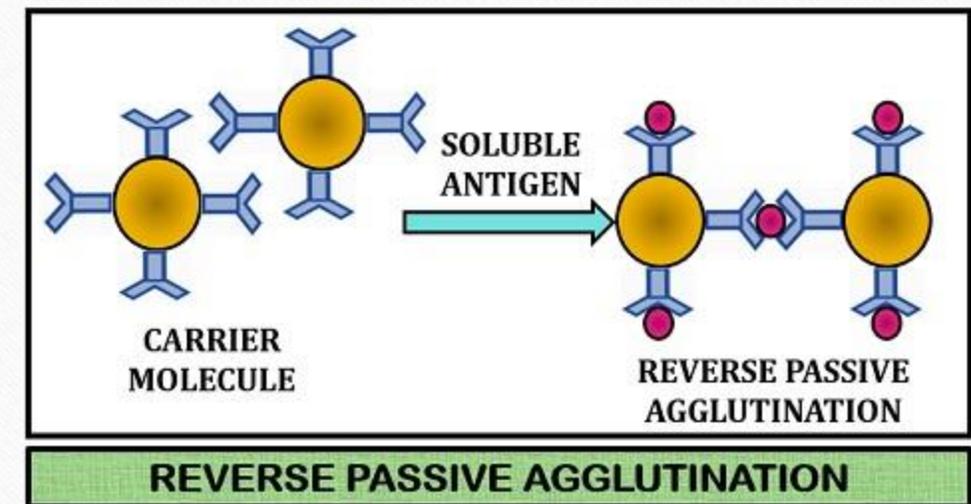
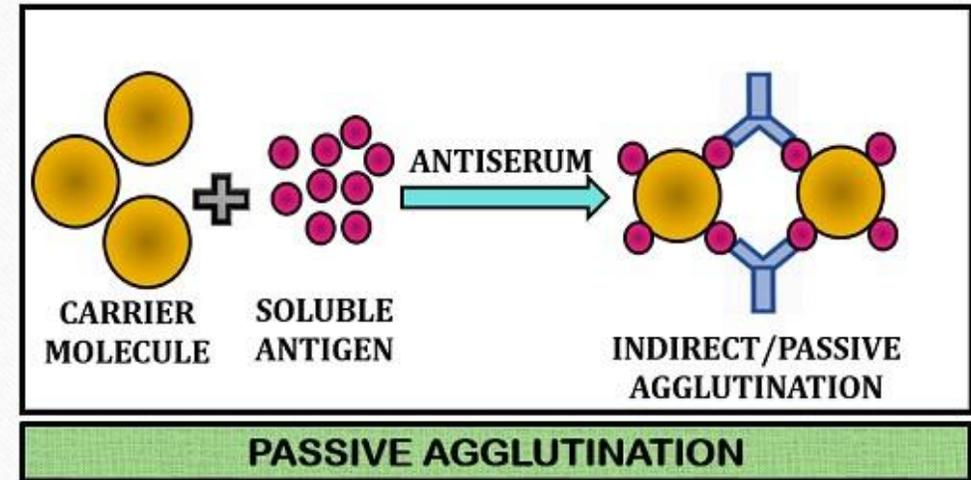
3. Reverse Passive agglutination.

Principle

- Antigen binds to soluble **antibody coated on carrier** particles and results in agglutination.
- Detects antigens.

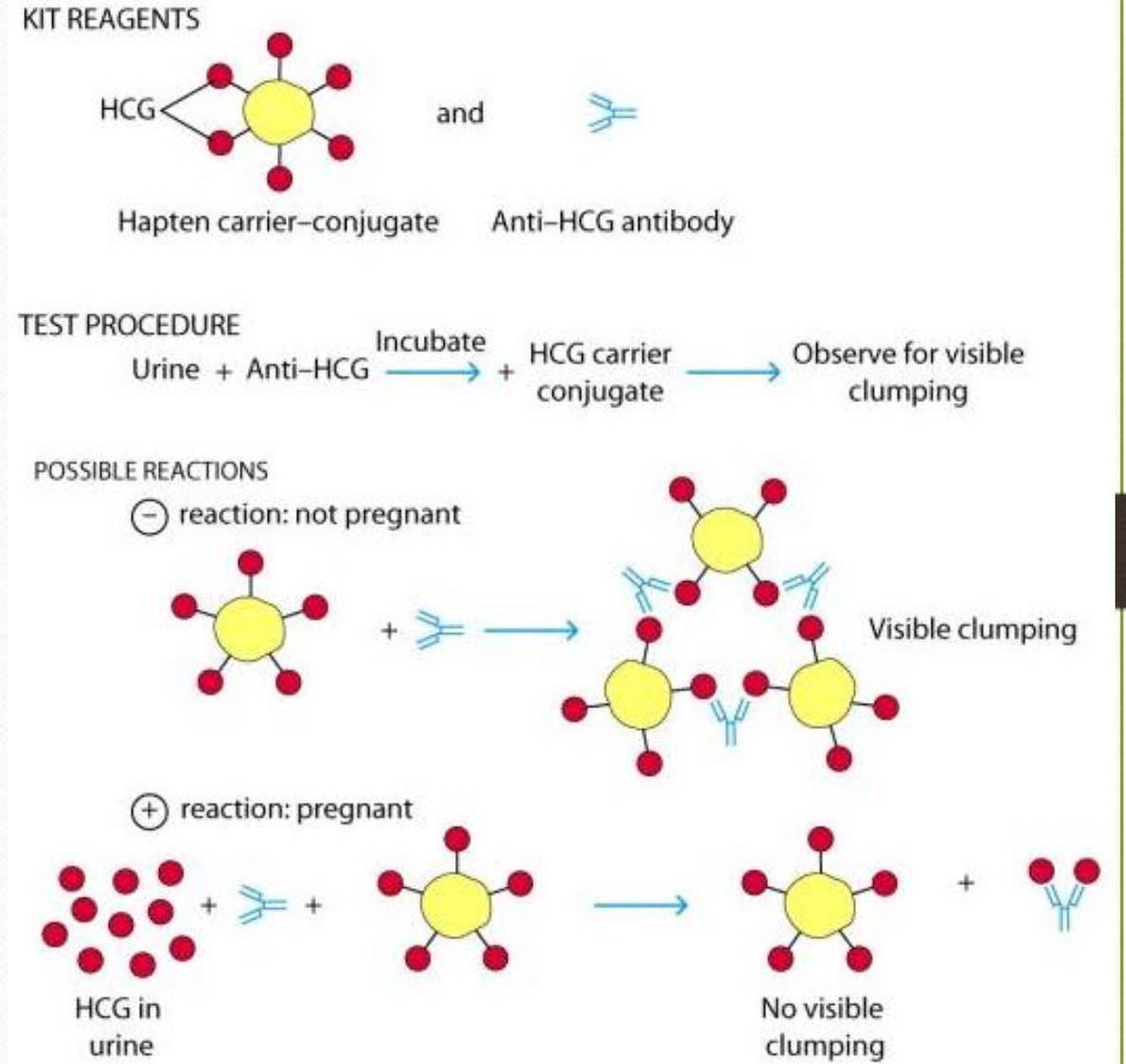
Example

- Detecting cholera toxin.



4. Agglutination inhibition

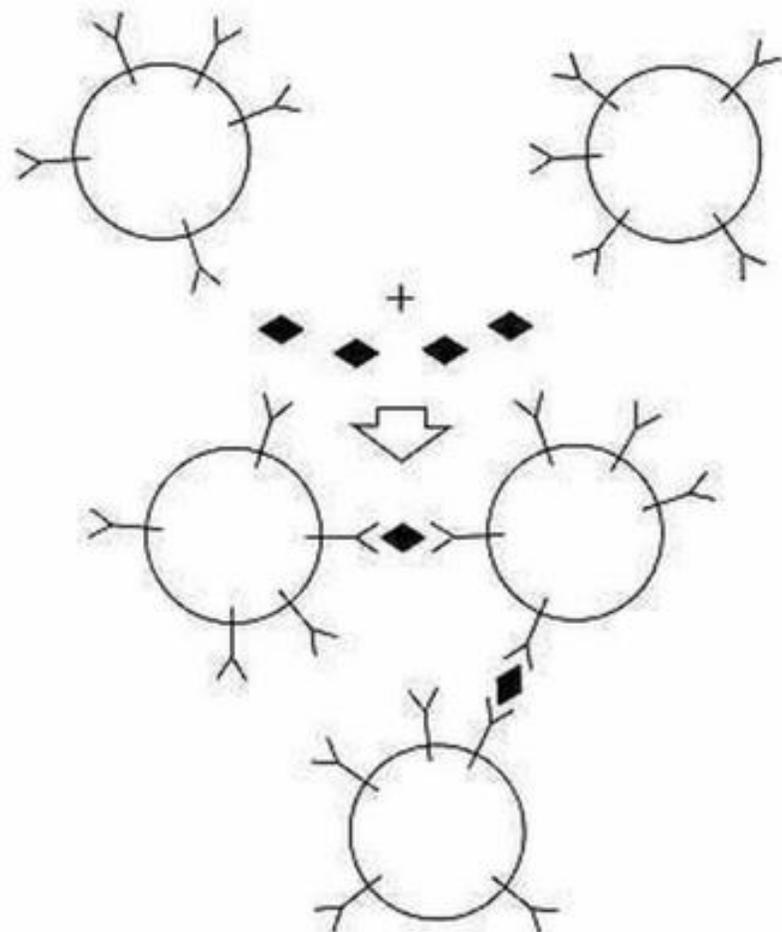
- Agglutination inhibition reactions are based on **competition** between particulate and soluble antigens for limited antibody-combining sites.
- The lack of agglutination is an indicator of a positive reaction.



5. Coagglutination

- Coagglutination (CoA) is similar to the Latex Agglutination technique for detecting antigen.
- Protein A, a uniformly distributed cell wall component of *Staphylococcus aureus*, is able to bind to the Fc region of most IgG isotype antibodies leaving the Fab region free to interact with antigens present in the applied specimens.
- The visible agglutination of the *S. aureus* particles indicates the antigen-antibody reaction.

Coagglutination



Specific antibody
bound to particles

+

Specific antigen

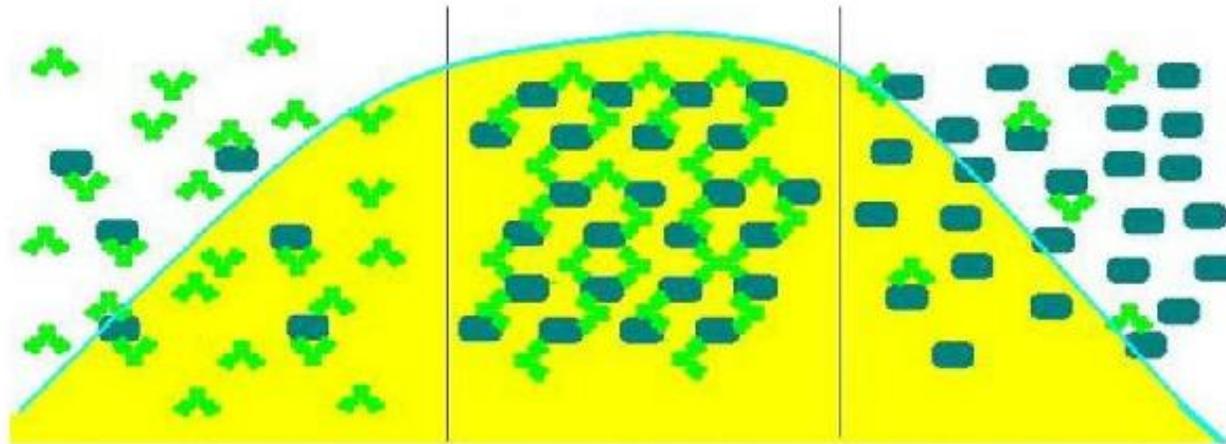
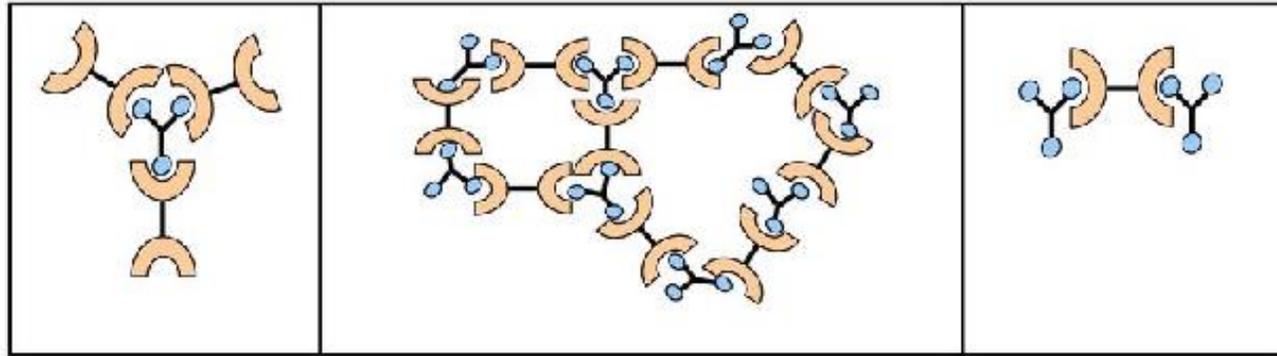


Coagglutination

B. Precipitation reaction

- Amount of precipitate Influenced by - Relative proportions of Ag & Ab
- Maximum precipitation occurs when Ags & Abs at optimal or equivalent proportions.
- Precipitation occur when a lattice (line or lattice) formed.
- ***Prozone phenomenon:*** antibody excess, no lattice network is formed.
- ***Postzone phenomenon:*** antigen excess. no lattice network is formed.
- ***for precipitation reactions to be detectable, they must be run in the zone of equivalence.***

Precipitation Curve



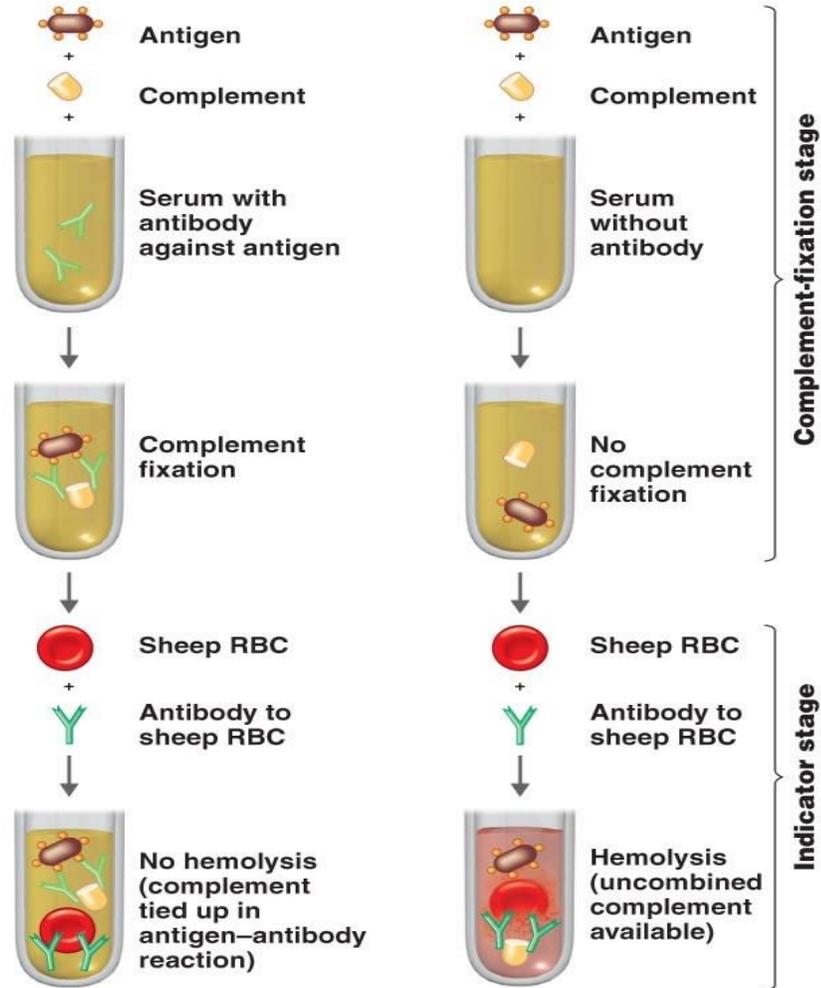
Ab excess

**Equivalence -
Lattice formation**

Ag excess

C. Complement fixation test

- The complement fixation test is an immunological medical test looking for evidence of infection. It tests for the presence of either specific antibody or specific antigen in a patient's serum.
- It uses sheep red blood cells (sRBC), anti-sRBC antibody and complement, plus specific antigen (if looking for antibody in serum) or specific antibody (if looking for antigen in serum).
- If either the antibody or antigen is present in the patient's serum, then the complement is completely utilized, so the sRBCs are not lysed. But if the antibody (or antigen) is not present, then the complement is not used up, so it binds anti-sRBC antibody, and the sRBCs are lysed.



(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.

Factors that affect agglutination reaction

1. Antigen-Antibody Concentration.
2. Antibody Size.
3. Number of Antigen Combining Sites on the Antibody.
4. Number of Antigenic Sites on the Red Cell.
5. Location of Antigenic Sites on the Membrane.
6. Zeta Potential.
7. Chemical Forces Affecting Antigen - Antibody Reactions
8. pH
9. Temperature
10. Ionic Strength
11. Time



Blood type....



Blood type....



Blood type....



Blood type....



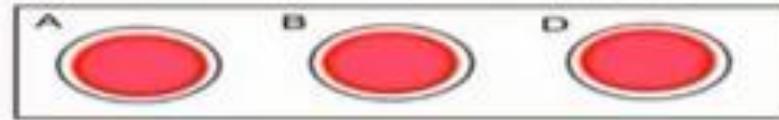
Blood type....



Blood type....



Blood type....



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