

**Serological tests 1**  
**(Antigen antibody interactions)**  
**Lab 2**

---

**Mathhar Ahmad Abu morad M.Bio**

**Department of Microbiology and immunology**

**Faculty of Medicine, Mu'tah 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

## Characteristics of this reaction

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



- Specific
- Optimum temp. 37-56c.
- High affinity
- Non- covalent interactions



□ An **immunoassay** is a method of target (detection/quantitation) antigen (or antibody) capture in samples using a specific antibody (or antigen),

1. Precipitation; Soluble antigen + specific antibody = Insoluble Precipitate of Ag-Ab complex (IGG)
2. Agglutination; if the antigen is insoluble or cell/ tissue bound and Ab is soluble (IGM, IGA)
3. Complement fixation (CFT); if the antibody in patient serum unites specific antigen, this consume complements in the test so no lysis of the the indicator sheep RBC (positive)
4. radio-immunoassays (radioisotopes)

5. ELISA; enzyme-linked immunosorbent assay is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones using an antibody linked to enzyme react with a substrate give color.

6. Western blot . In this technique a mixture of proteins is separated based on molecular weight through electricity (gel electrophoresis). These results are then transferred to a membrane producing a band for each protein, the specific protein is identified by binding specific radiolabeled or enzyme linked antibody.

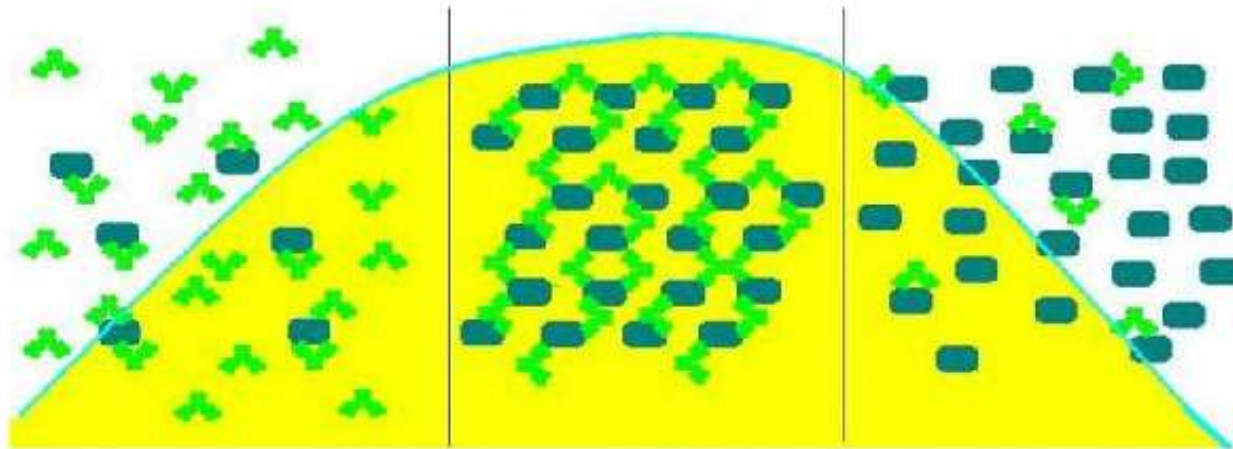
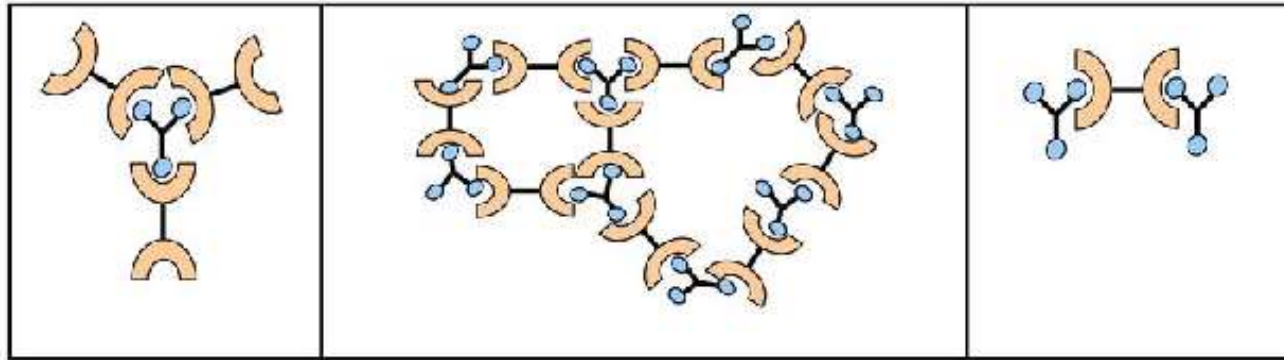
- In Ag-Ab binding; **Precipitation**, Precipitation reactions are based on the interaction of antibodies and antigens. They are based on **two soluble antigen and antibody** that come together to make one insoluble product, the precipitate which appear as line between 2 solutions.
- In Ag-Ab binding, **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.



# A. Precipitation reaction

- Amount of precipitate Influenced by - 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**

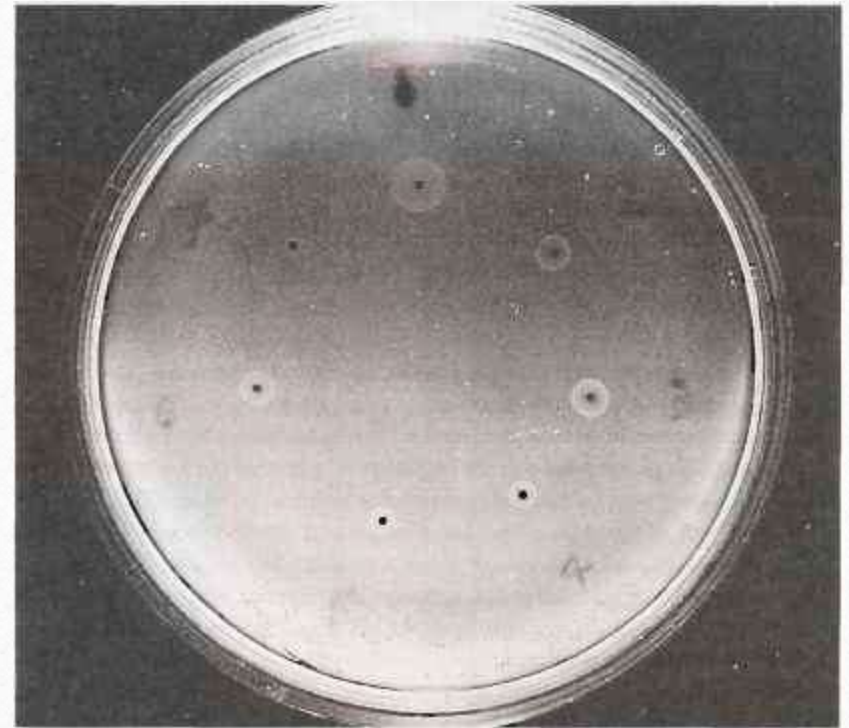
**Ag excess**



## 1-Single radial diffusion On a slide

Single diffusion in two dimensions (Radial immunodiffusion))

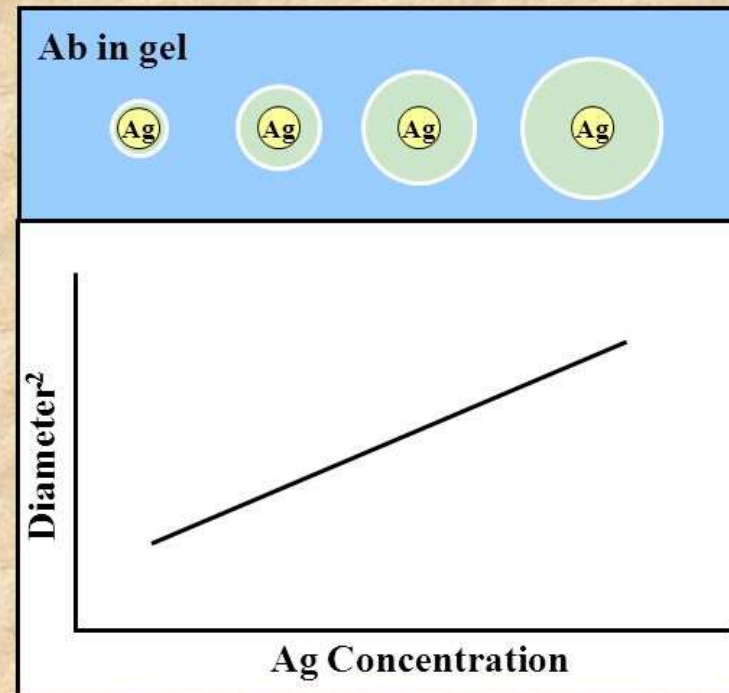
- Ab incorporated in agar gel
- Ag. added to wells in agar.
- Ag. diffuses radially from the well
- Forms precipitation ring around antigen
- Diameter of halo – estimate of conc. of Ag.
- Used for estimation of Igs levels in serum, screening Abs against viruses (Influenza) or bacteria



# Precipitation reaction

## Radial Immunodiffusion (Mancini)

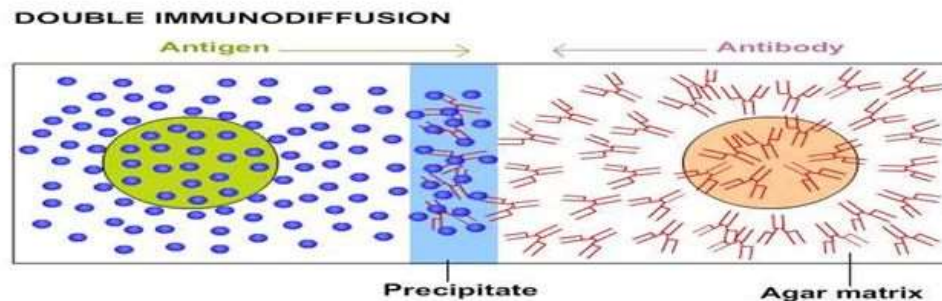
- Method
  - Ab in gel
  - Ag in a well
- Interpretation
  - Diameter of ring is proportional to the concentration
- Quantitative
  - Ig levels





## 2- Double Immunodiffusion

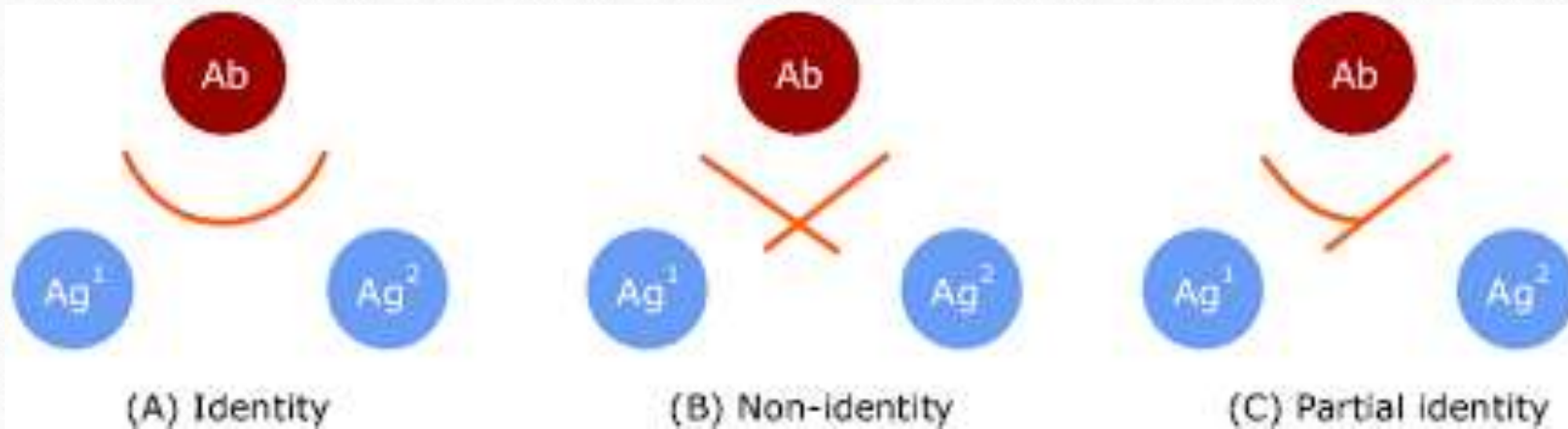
- Diffusion of antibody and antigen towards each other in an Agarose gel.
- A line of precipitate will form if the antibody binds to antigen.
- Used to determine if an antigen or antibody is present





# Ouchterlony results types to detect microbial antigens

---



## B. agglutination reactions

These reactions take part in two stages,

- **Sensitization**, the antibody binds to the red cell( Antigens) or sensitizes it.
  - **Agglutination**. In the second stage, the sensitized red cells (Antigens) agglutinate.
1. **Direct:** are commonly used in various diagnostic and serological assays, such as blood typing (hemagglutination), bacterial agglutination tests, and other immunological tests where the primary goal is to detect the presence of specific antibodies in the patient's sample by directly mixing it with the target antigen. In this reaction, antibodies in the serum cause the agglutination of red blood cells that express the corresponding antigen on their surface.
  2. **Indirect:** In this reaction, red blood cells are coated with soluble antigens, and antibodies in the serum cause agglutination when they bind to these antigen-coated cells.

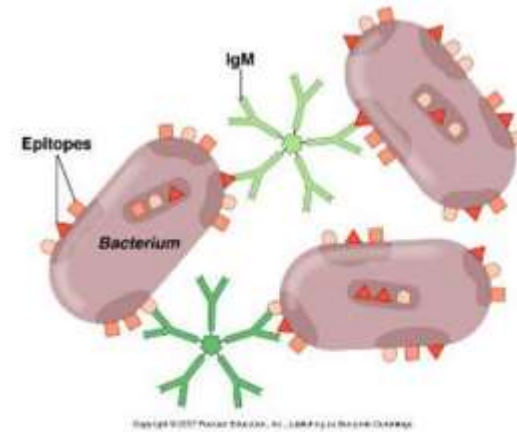
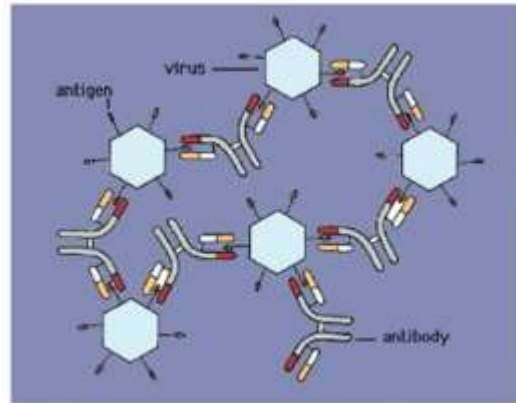


- The general term **agglutinin** is used to describe antibodies that agglutinate particulate antigens (**agglutinogen**).

When the antigen is an erythrocyte the term **hemagglutination** 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*)





# 1. Direct Agglutination (Active)

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

---

## **A. Slide Agglutination:**

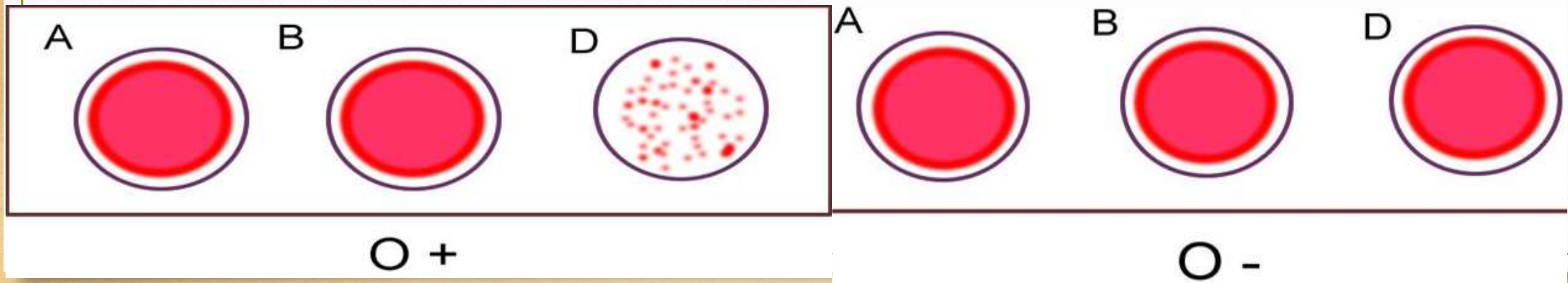
In this method, blood samples are mixed with Anti-A, Anti-B, and Anti-D antibody on a slide to perform the agglutination.

## **Applications:**

- Used for blood grouping
- Used for the identification of bacteria from clinical specimens.

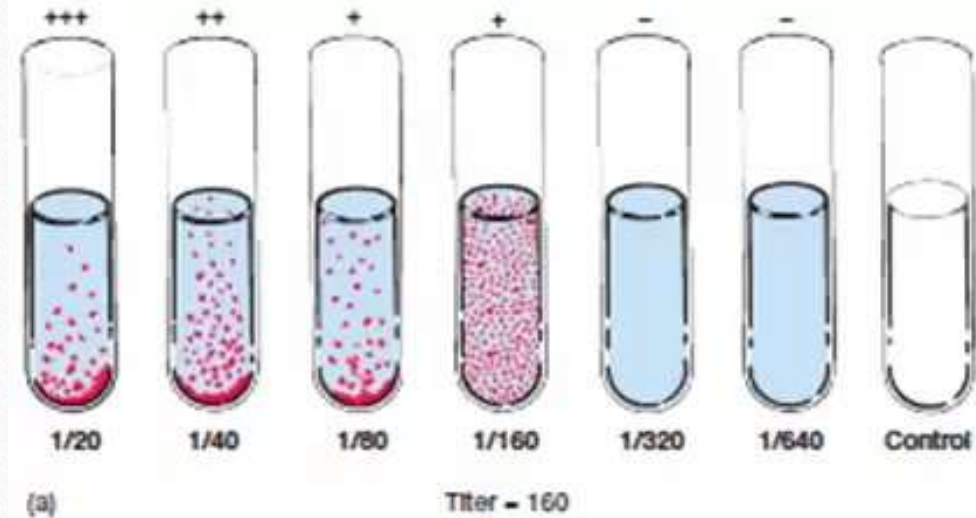
## Example:

- Uses of hemagglutination are Blood grouping & Cross matching, Antisera of the IgM type can be used 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 Typhoid fever to detect specific Ab.
- 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

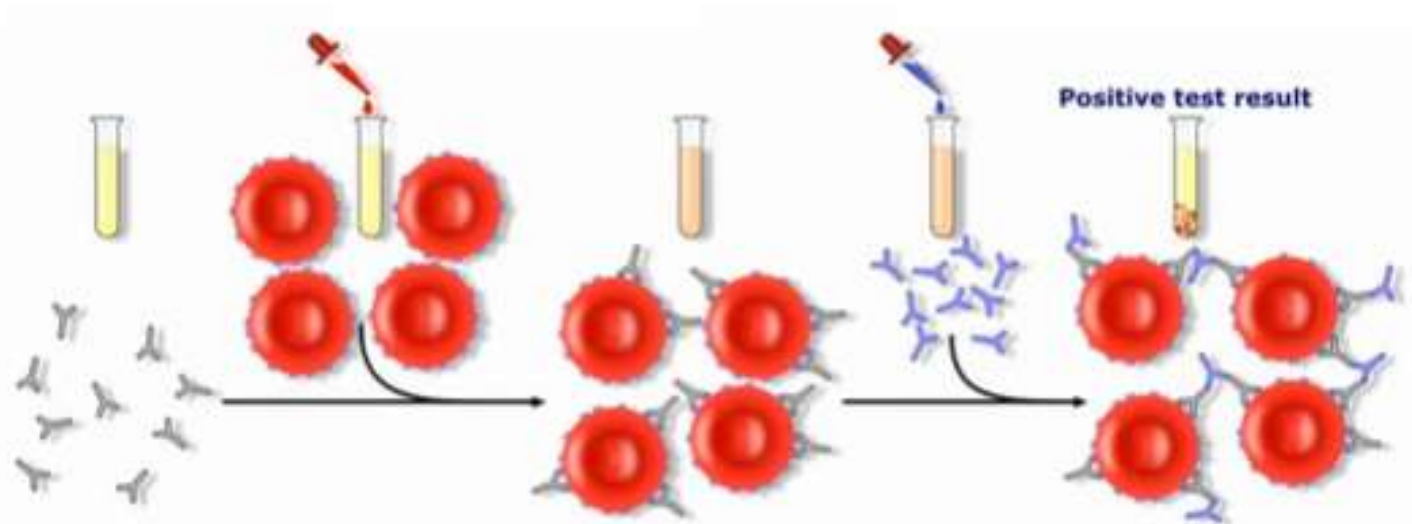
Patient	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Pos.	Neg.	Titer
1	●	●	●	●	●	●	○	○	○	○	●	○	64
2	●	●	●	○	○	○	○	○	○	○	●	○	8
3	●	●	●	●	●	●	●	●	●	○	●	○	512
4	○	○	○	○	○	○	○	○	○	○	●	○	<2
5	●	●	●	●	●	○	○	○	○	○	●	○	32
6	○	○	●	●	●	●	●	○	○	○	●	○	128
7	●	●	●	●	●	○	○	○	○	○	●	○	32
8	●	●	○	○	○	○	○	○	○	○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

A high titer (highest dilution) suggests a strong immune response, while a low titer may indicate a weaker response.



- Anti-human globulin (AHG), also known as Coombs reagent, is a type of antibody used in immunohematology and blood bank testing.
- The Indirect Coombs Test is used in pre-transfusion testing to determine if the patient's serum contains any antibodies that could react with the donor's red blood cells. This is essential to prevent transfusion reactions in patients.
- This is often seen in autoimmune hemolytic anemia or hemolytic disease of the newborn.

## Indirect combs' test



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

### 3. Reverse Passive agglutination.

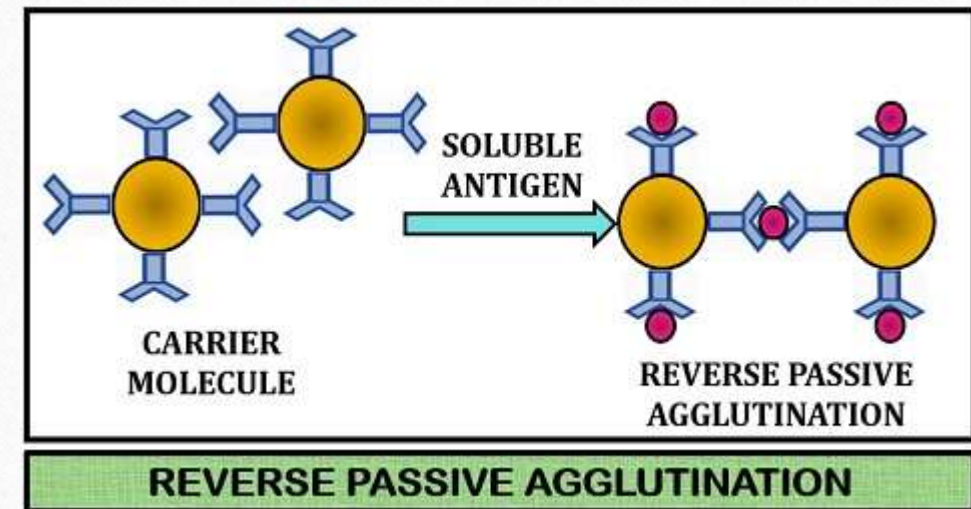
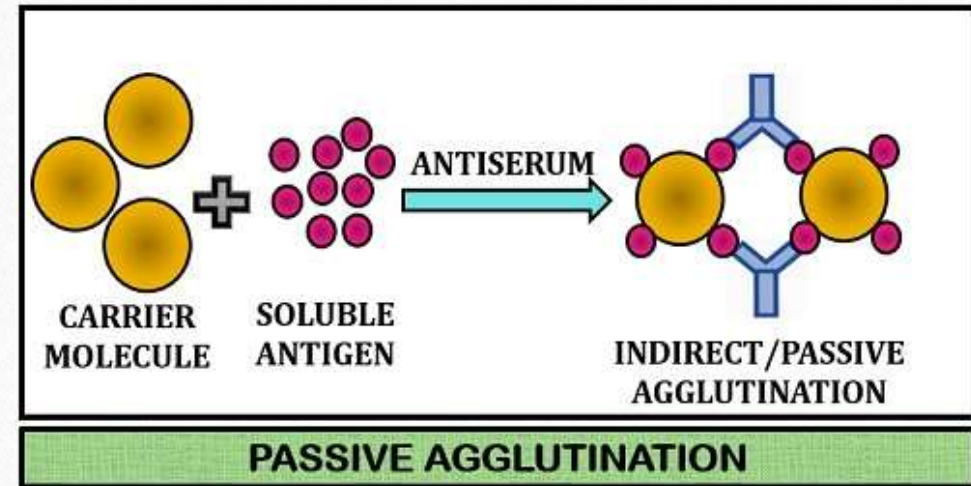
#### Principle

- Soluble Antigen binds to **antibody coated on carrier** particles and results in agglutination.

- Detects antigens.

#### Example

- Detecting cholera toxin.





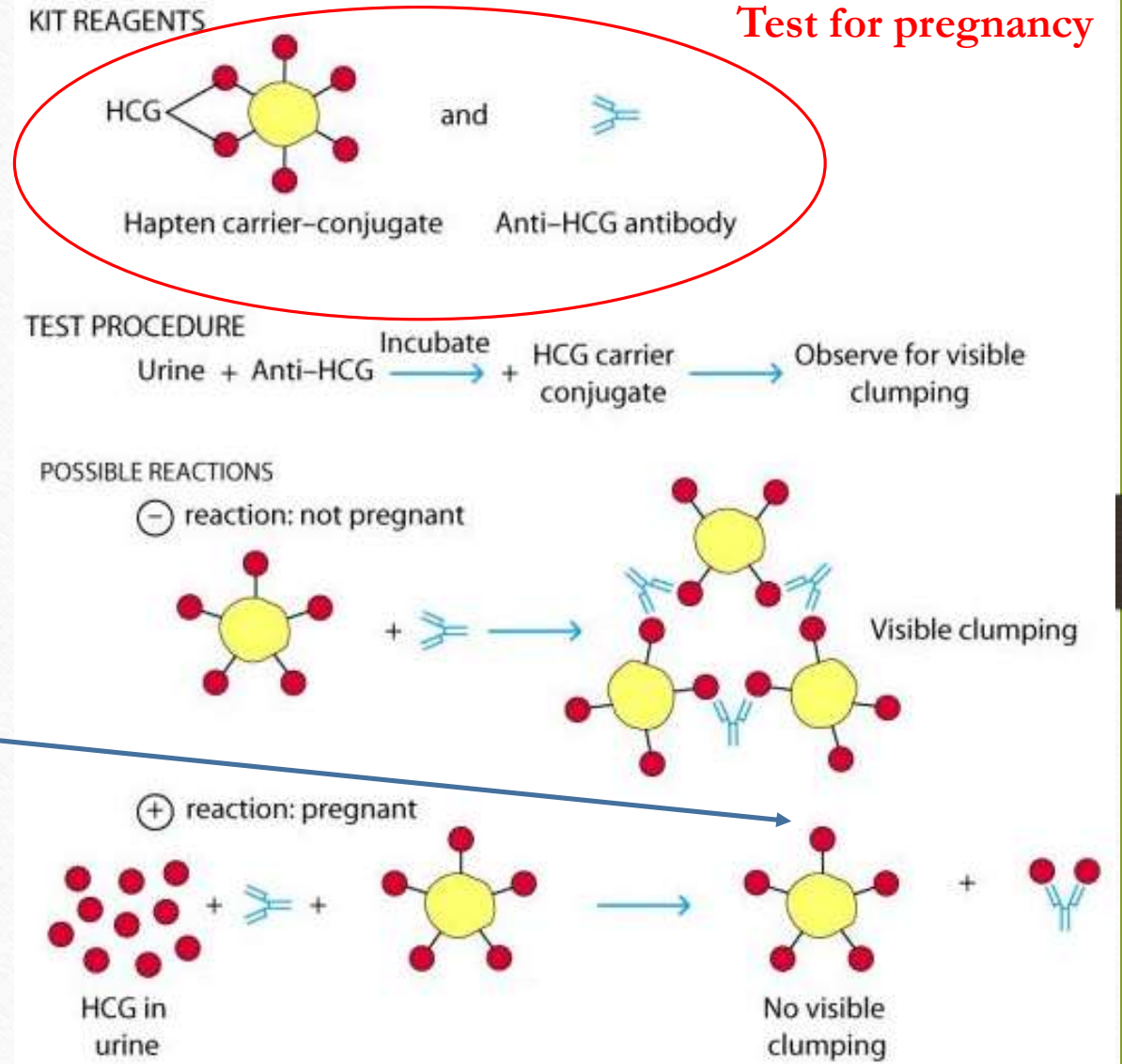
## 4. Agglutination inhibition

- 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.

Other examples:

- RF**: Rheumatoid factor.
- CRP**: C-reactive protein in inflammation test.

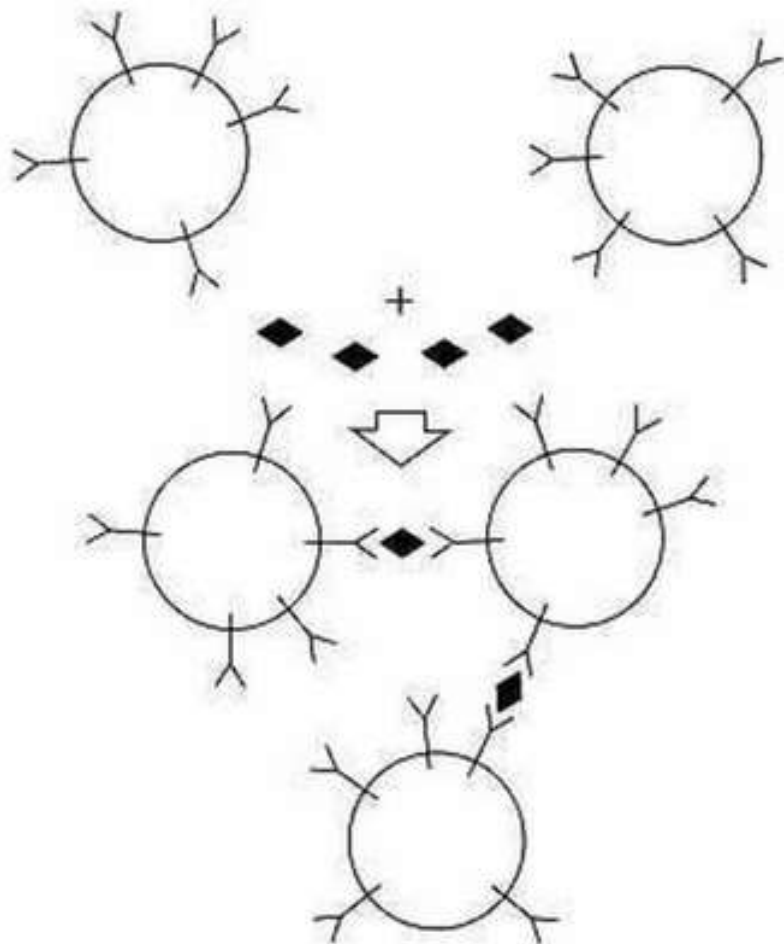


## 5. Coagglutination

- 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

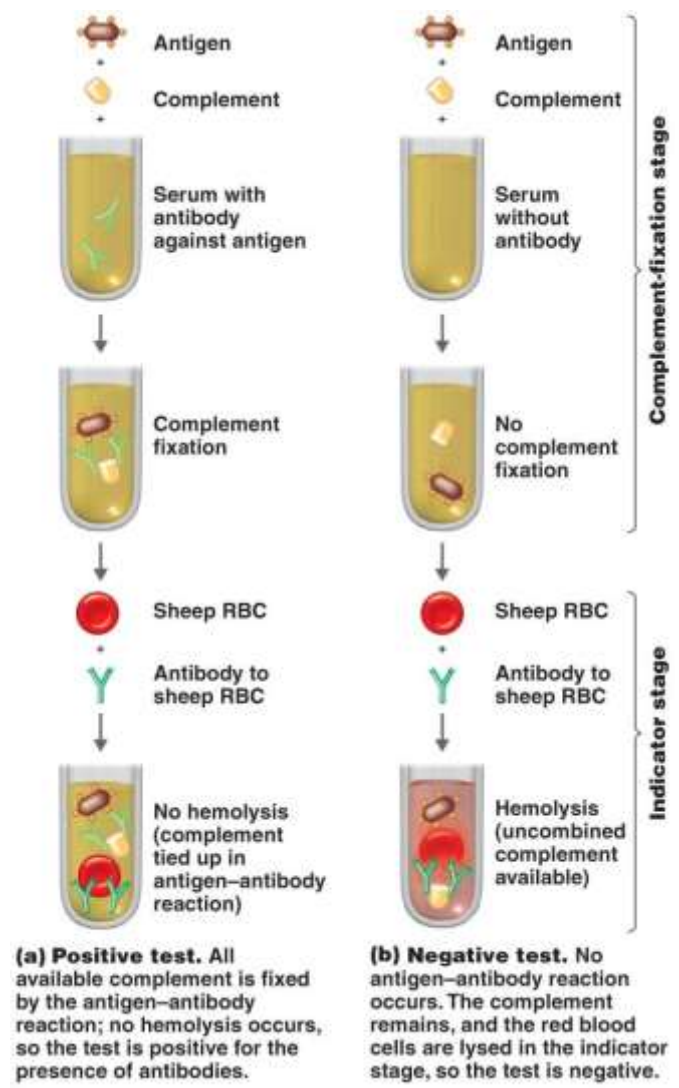
## C. Complement fixation test

- 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.

---

- Complement is a group of proteins that are normally present in blood serum and play a role in immune defense.
- By using an indicator system, sheep red blood cells (sRBC) coated with antibodies that specifically bind to complement proteins (hemolysin), 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.





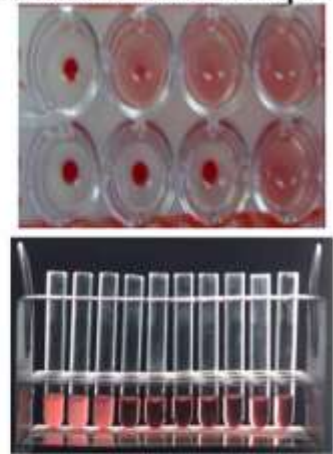
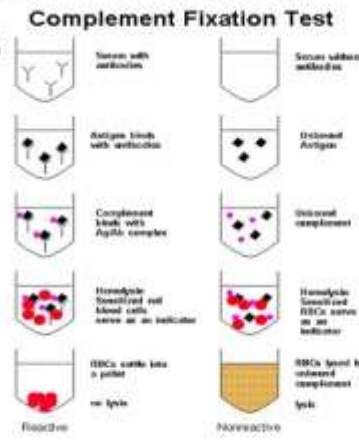
## Complement fixation test(CFT)

Serum(? Abs)+ known Ag+C →Incubate for 1 hour, then add indicator system(Sheep RBCs+Ani-sheep RBCs).→Incubate for 1 hour

+ve = No lysis  
-ve = lysis

### Uses:

Diagnosis of:  
Syphilis, Viral Infections, Gonorrhoea,....



# Uses of CFT

To diagnose

- syphilis (Wassermann reaction)
- Gonorrhoea
- Rickettsial infection
- screening of antibodies against a variety of possible pathogenic microbes (especially viruses)

Positive case with titer 64



No Hemolysis

Hemolysis

Negative case



Hemolysis