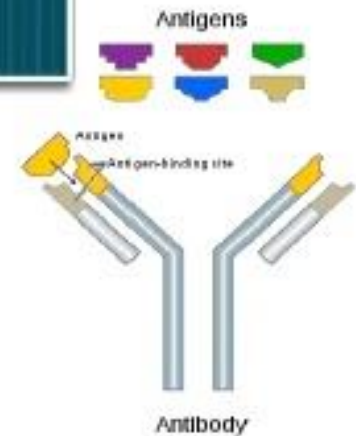


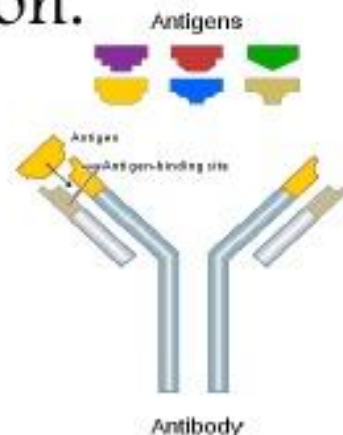
# Antigen and Antibody Reaction

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## INTRODUCTION:

- The antigens and the antibodies combine specifically with each other. This interaction between them is called Antigen-Antibody reaction.
- It may be abbreviated as Ag – Ab reaction.
- These form the basis for humoral immunity or antibody mediated immunity.
- These reactions form the basis for detection of infectious disease causing agents and also some non-specific Ag's like enzymes.



- When Ag – Ab reactions occur invitro, they are known as serological reactions.

- The reactions between Ag and Ab occur in three stages.

- In first stage the reaction involves formation of Ag-Ab complex.

- The second stage leads to visible events like precipitation, agglutination etc.

- The third stage includes destruction of Ag or its neutralization

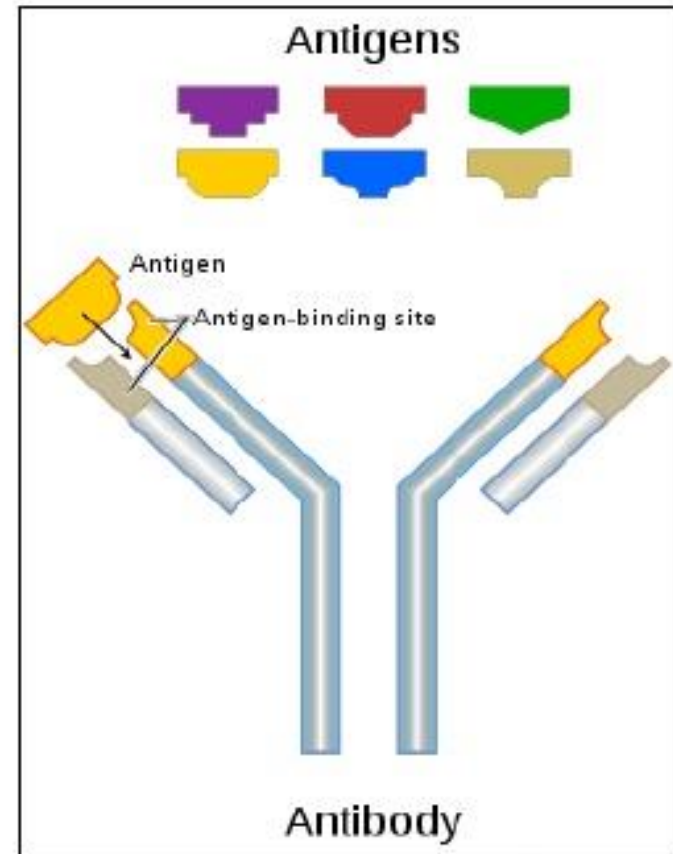


## **Salient Features of Antigen – Antibody Reaction:**


- **Specificity of Antigen – Antibody Reaction.**
- **Immune complex.**
- **Binding Site of Antigen – Antibody Reaction.**
- **Binding Force of Antigen – Antibody Reaction.**

# Specificity of Antigen – Antibody Reaction:

• Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen.



**Each antibody binds to a specific antigen; an interaction similar to a lock and key.**



- For example, the antibody produced against lens antigen will react only with lens-antigen. Similarly, the antibody produced against kidney antigen will react with only kidney- antigen. A standard lock can be opened by its own key only as one antibody can react with its own antigen.

### **Immune Complex:**

- An immune complex is formed from the integral binding of an antibody to a soluble antigen.
- The bound antigen acting as a specific epitope, bound to an antibody is referred to as a singular immune complex.



- Mechanisms of antigen-antibody interaction leading to inflammation. Antigen-antibody immune complex formation results in complement activation, opsonization of target cells, assembly of membrane attack complexes and release of complement activators for chemotaxis.
- Fc receptor mediated cell activation triggers cellular responses, such as phagocytosis, antibody-dependent cellular cytotoxicity (ADCC) and release of inflammatory mediators.

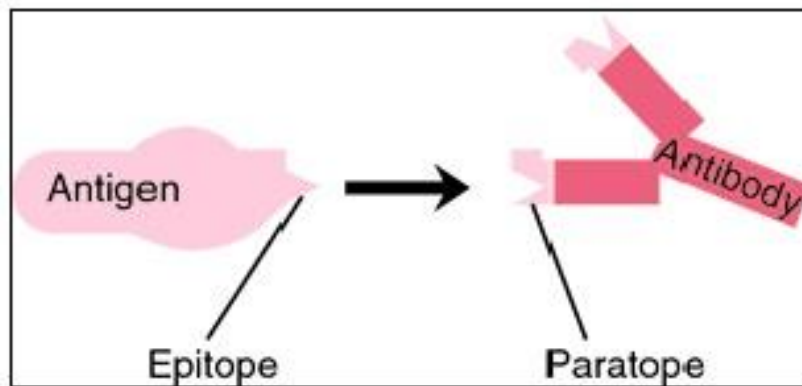


**Antigen – Antibody Reaction**



## Binding Site of Antigen – Antibody Reaction:


- In antigen - antibody reaction, the antibody attaches with the antigen.
- The part of antigen which combines with antibody is called Epitope.
- An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.
- The part of an antibody that recognizes the epitope is called a paratope.



## Antigen and Antibody to Show Epitope And Paratope

### Binding Force of Antigen – Antibody Reaction:

- The binding between antigen and antibody in ag – ab reaction is due to three factors namely:
  - Closeness between antigen and antibody.
  - Non – covalent bonds or Intermolecular forces .
  - Affinity of antibody.

- 
- Closeness between antigen and antibody: When antigen and antibody are closely fit, the strength of binding is great. When they are apart binding strength low.
  - Non – Covalent Bonds: The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds.
  - Affinity of antibody: Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody.



- A strong antigen – antibody interaction depends on a very close fit between the antigen and antibody which requires high degree of specificity.

## **Properties of Antigen – Antibody Reaction:**

The properties of antigen and antibody can be explained with the help of three points. They are:

- **Antibody Affinity.**
- **Antibody Avidity**
- **Cross reaction.**

## Affinity of Antibody:

Interactions between antigen and antibody involve non-covalent binding of an antigenic determinant (epitope) to the variable region (complementarity determining region, CDR) of both the heavy and light immunoglobulin chains. These interactions are analogous to those observed in enzyme-substrate interactions and they can be defined similarly. To describe the strength of the antigen-antibody interaction, one can define the affinity constant ( $K$ ) as shown:

$$\text{Affinity } K = \frac{[\text{Ab} - \text{Ag}]}{[\text{Ab}] [\text{Ag}]} = 10^4 \text{ to } 10^{12} \text{ L/mol}$$

## **Avidity of Antibody:**

- It is the strength of the bond after the formation of Ag-Ab complexes.
- It is used to denote the overall capacity of antibodies to combine with the multivalent antigen.
- A multivalent Ag has many types of antigenic determinants.
- When injected into the blood, each antigenic determinant stimulates the production of a particular antibody.

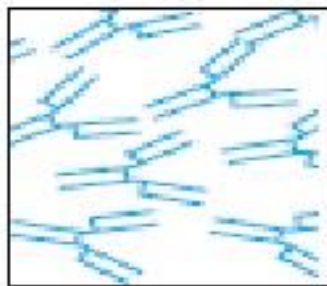


## **Types of Antigen – Antibody Reaction:**

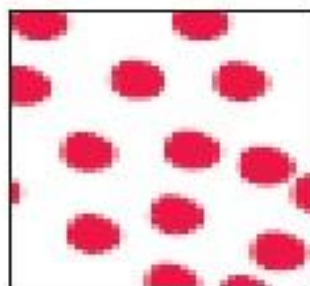
- The types of antigen – antibody reactions are:
  - **Precipitation Reaction.**
  - **Agglutination Reaction.**
  - **Complement Fixation.**
  - **ELISA – Enzyme Linked ImmunoSorbent Assay.**
  - **Immunofluorescence.**

## Precipitation Reaction:

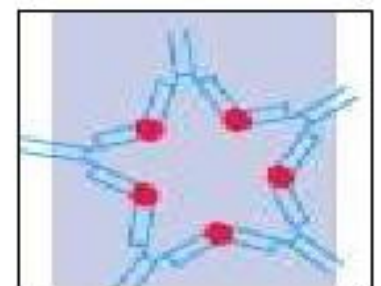
When a soluble Ag combines with its Ab in the presence of an electrolyte (NaCl) at a particular temperature and pH, it forms an insoluble precipitate of Ag-Ab complex. The Ab causing precipitation is called Precipitin and the reaction is called as precipitation reaction.



**Antibodies**



**Antigens**

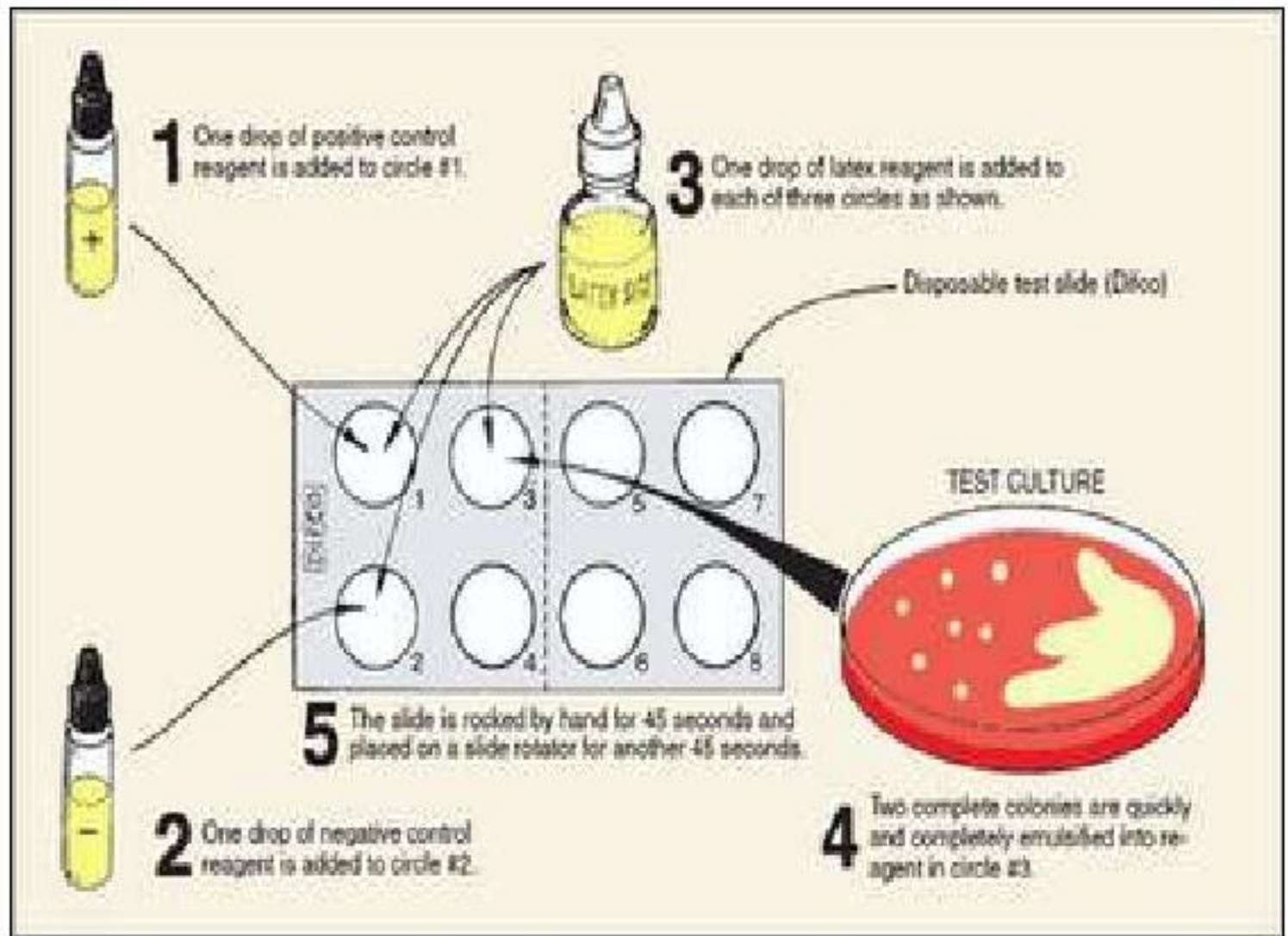


**Ag-Ab complex**

## Agglutination Reaction:

- When a particular Ag is mixed with its Ab's in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.
- The Ab of the serum causes the cellular Ag's to form clumps and these are called Agglutinins.
- The particulate antigens that are aggregated are termed Agglutinogens.
- **Slide agglutination:** This is a rapid method to determine the presence of agglutinating antibodies.





**Slide Agglutination**

## Complement Fixation:

- Lysis of RBC or bacteria requires some non-specific unstable components of fresh serum which are called complement.
- This complement system comprises of 11 proteins and are present in every individual. They bind to Fc component of Ab involved in Ag-Ab complex. This ability of the Ag-Ab complex to fix complement is used in complement Fixation tests.
- In the first stage, the test Ag and the antiserum (heated to 56°C to inactivate complement) are mixed in the presence of known amount of complement. This is incubated at 4°C for 18h.

# ELISA – Enzyme Linked ImmunoSorbent Assay:

- In 1971, enzyme labeled Ag's and Ab's were developed as serological reagents for the assay of Ab's and Ag's.
- These are very simple, sensitive, economic and less hazard when compared to RIA.
- The ligand used here is a molecule which can detect the Ab and is covalently coupled to an enzyme such as peroxidase, betagalactosidase, alkaline phosphatase etc.