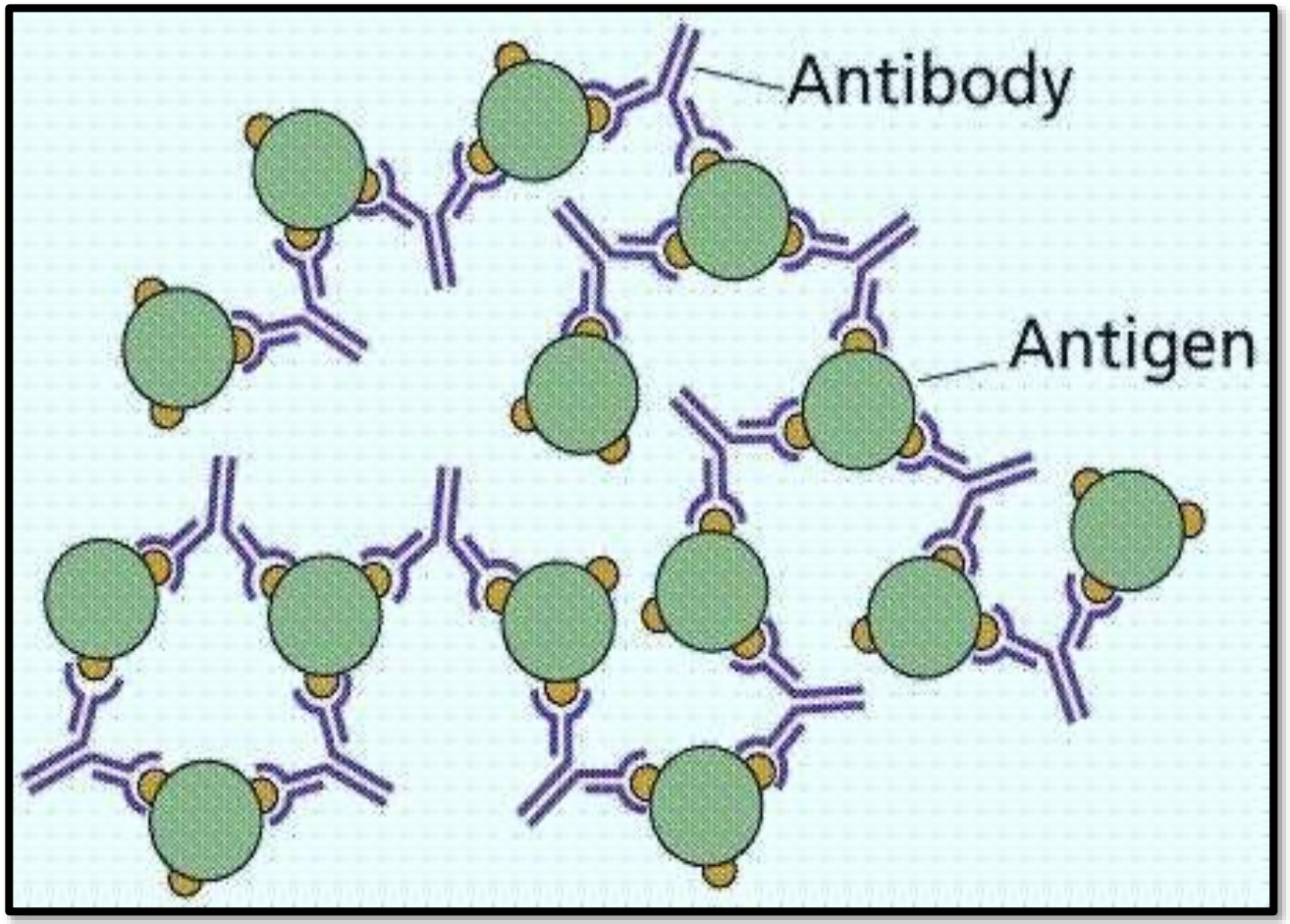
ANTIGEN & ANTIBODY REACTIONS



INTRODUCTION

Antigens & antibodies combine specifically with each other. This interaction between them is called 'Antigen-Antibody reaction'.

- Abbreviated as Ag – Ab reaction.

-They form the basis for humoral/antibody mediated immunity.

-They are used for detection of disease causing agents & some non-specific Ag's like enzymes.

-When Ag-Ab reaction occurs in-vitro they are known as '<u>serological reactions</u>'.

-The reactions b/w Ag & Ab occurs in 3 stages:

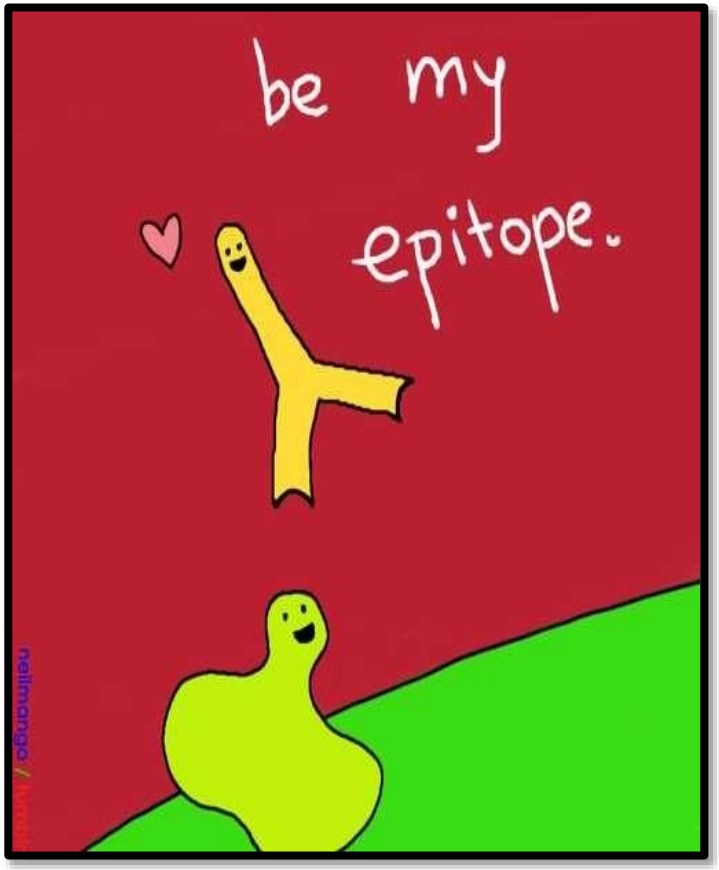
1st = formation of Ag-Ab complex.

2nd = leads to visible events like precipitation, agglutination etc.

3rd = destruction of Ag or its neutralization.

SALIENT FEATURES

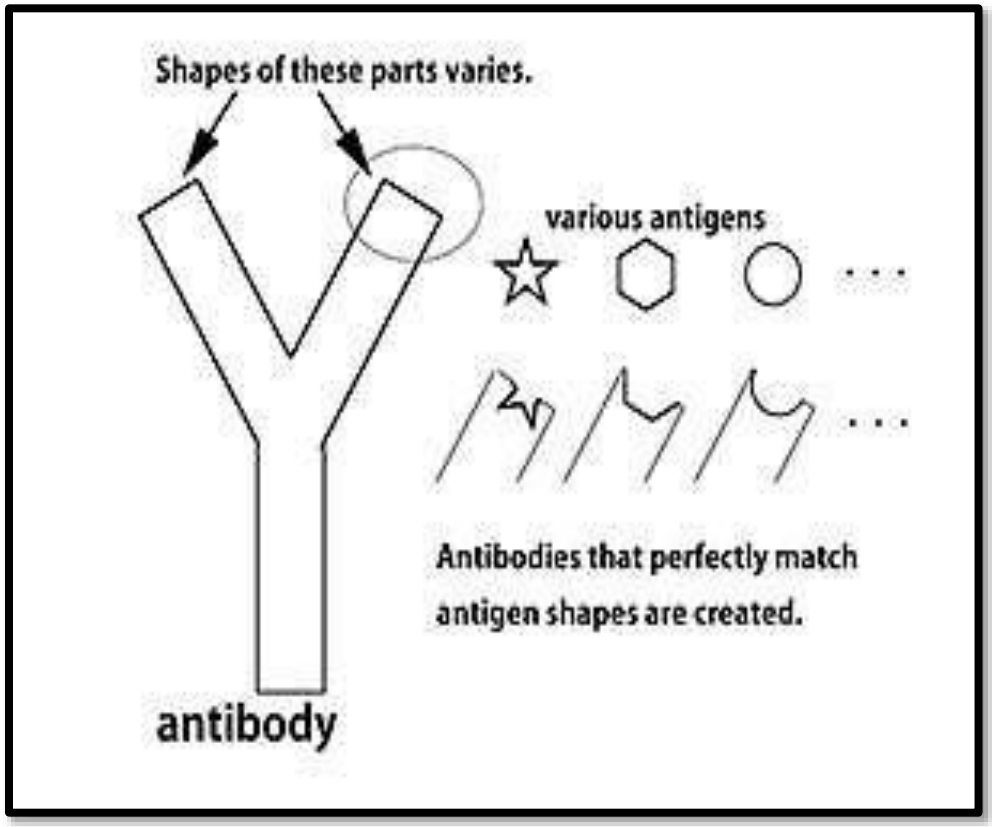
- Specificity.
- Immune complex.
- Binding Site of Antigen.
- Binding Force of Antigen.

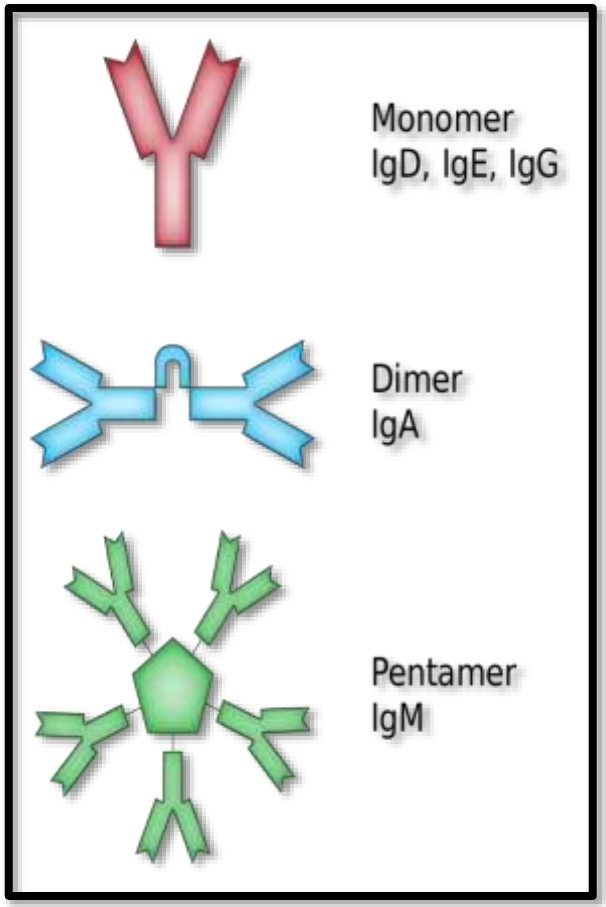


1. SPECIFICITY:

Refers to the ability of an individual antibody combining site to react with only one antigenic determinant (epitope).

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

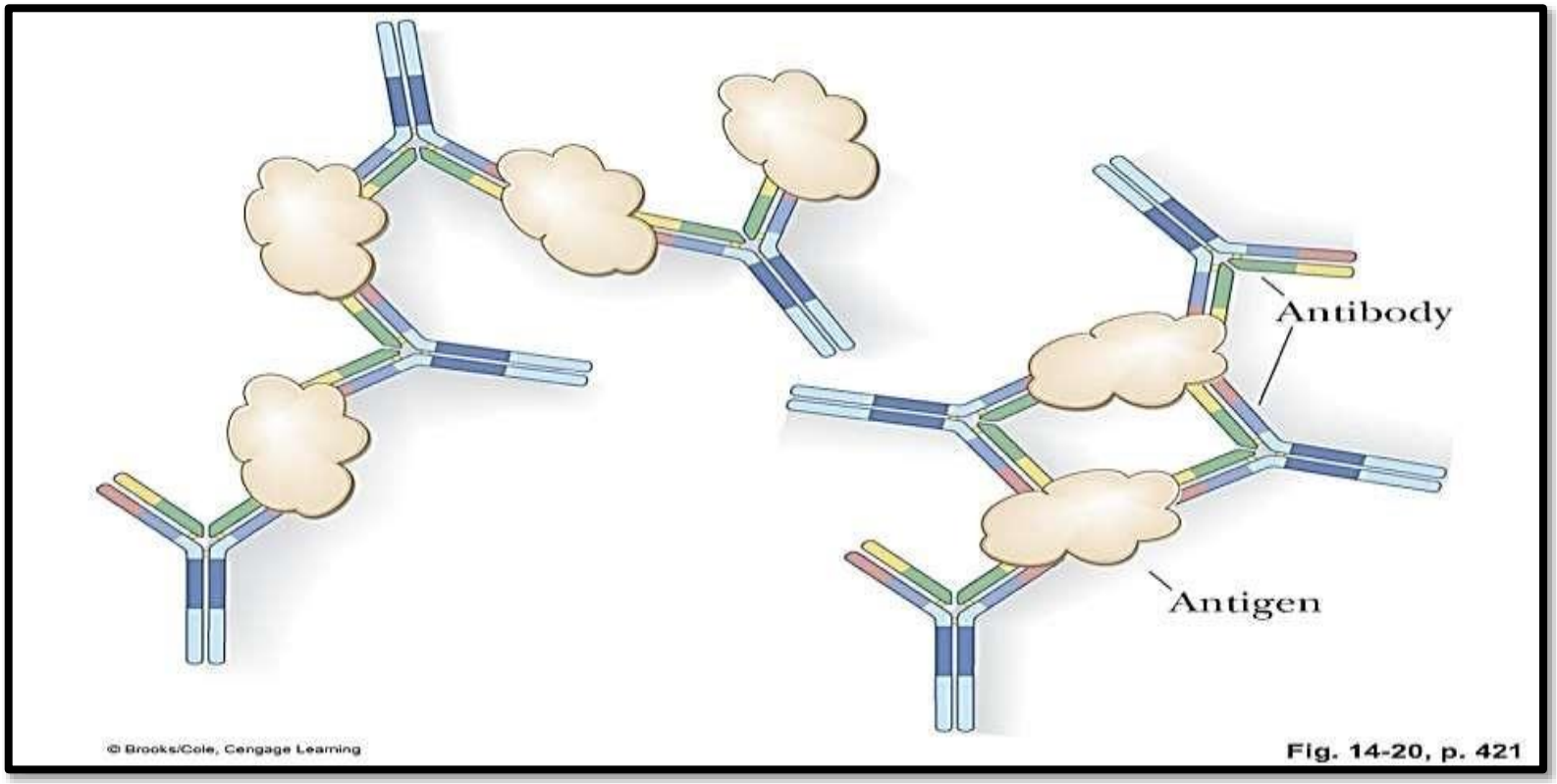




2.IMMUNE COMPLEX:

An immune complex is formed from the integral binding of an antibody to a soluble antigen.

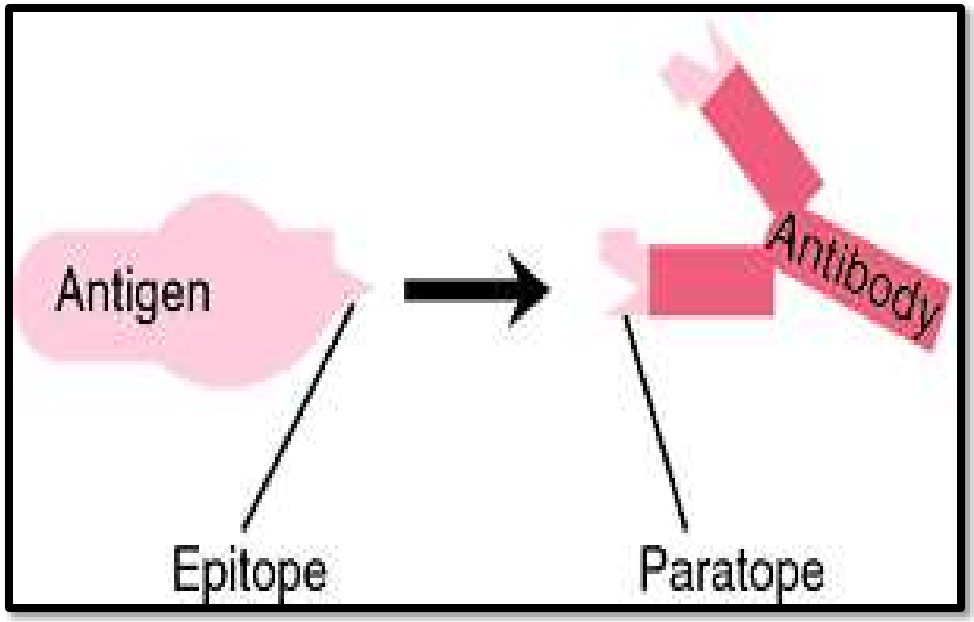
Ag + Ab → Ag-Ab complex

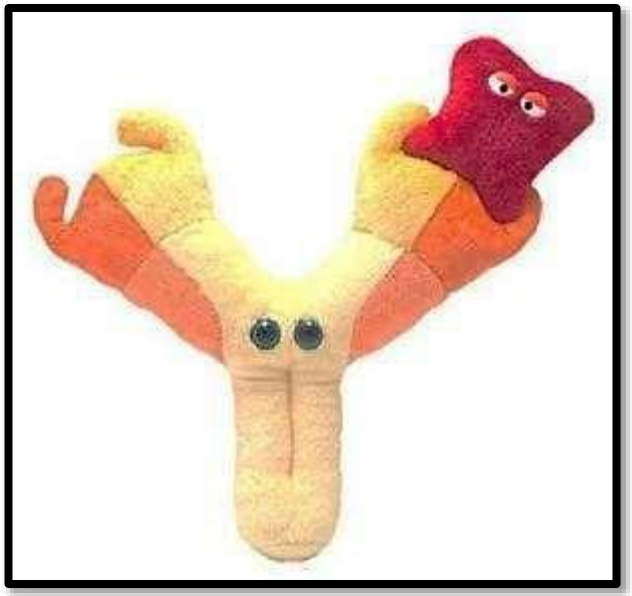


3. BINDING SITE OF Ag:

-The part of antigen which combines with antibody is called ‘Epitope’, recognized by the immune system, specifically by antibodies, B cells, or T cells.

-Part of an antibody that recognizes an epitope is called a ‘paratope’.





4. BINDING FORCE OF Ag:

- The binding b/w Ag & Ab in Ag – Ab reaction is due to three factors namely:

* Closeness b/w Ag & Ab -> more close = good strength of binding.

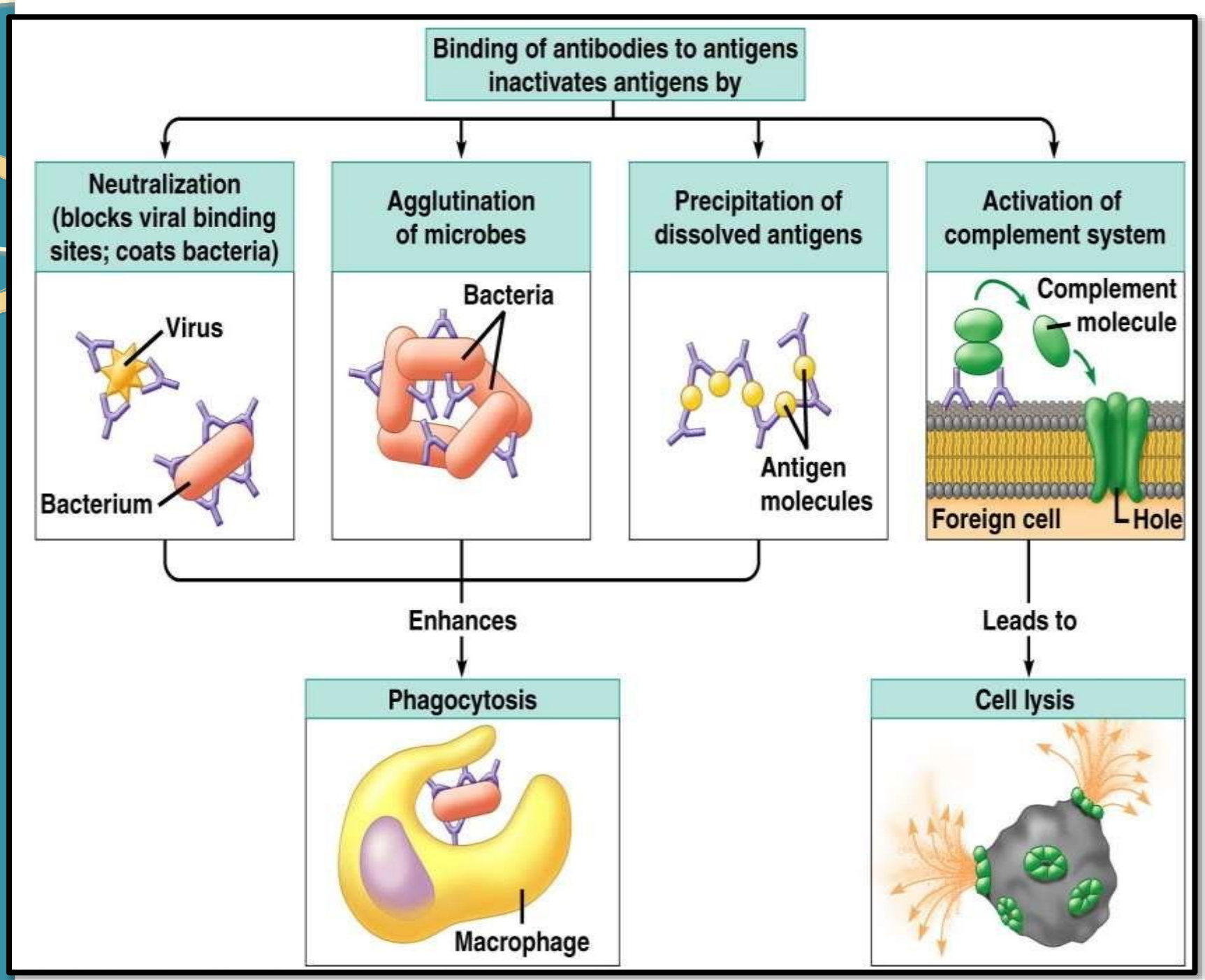
* Non – covalent bonds or Intermolecular forces -> hydrogen bonds, vander walls forces, hydrophobic bonds.

* Affinity of antibody -> strength of reaction b/w a single epitope & single paratope.

TYPES

The types of antigen-antibody reactions are:

- Precipitation Reaction.
- Agglutination Reaction.
- Complement Fixation.
- ELISA– Enzyme Linked ImmunoSorbent Assay.
- Immunofluorescence.


Binding of antibodies to antigens inactivates antigens by
Neutralization (blocks viral binding sites; coats bacteria)
Virus
Bacterium
Agglutination of microbes
Bacteria
Precipitation of dissolved antigens
Antigen molecules
Activation of complement system
Complement molecule
Foreign cell
Hole
Enhances
Leads to
Phagocytosis
Macrophage
Cell lysis

PRECIPITATION / IMMUNO-PRECIPITATION

The phenomenon of aggregation of sensitized antigen on addition of specific antibody (precipitin) to antigen in solution is called 'precipitation'.

- Precipitation occurs in two media:
    * Liquid
    * Gel

1. Precipitation in Liquids:

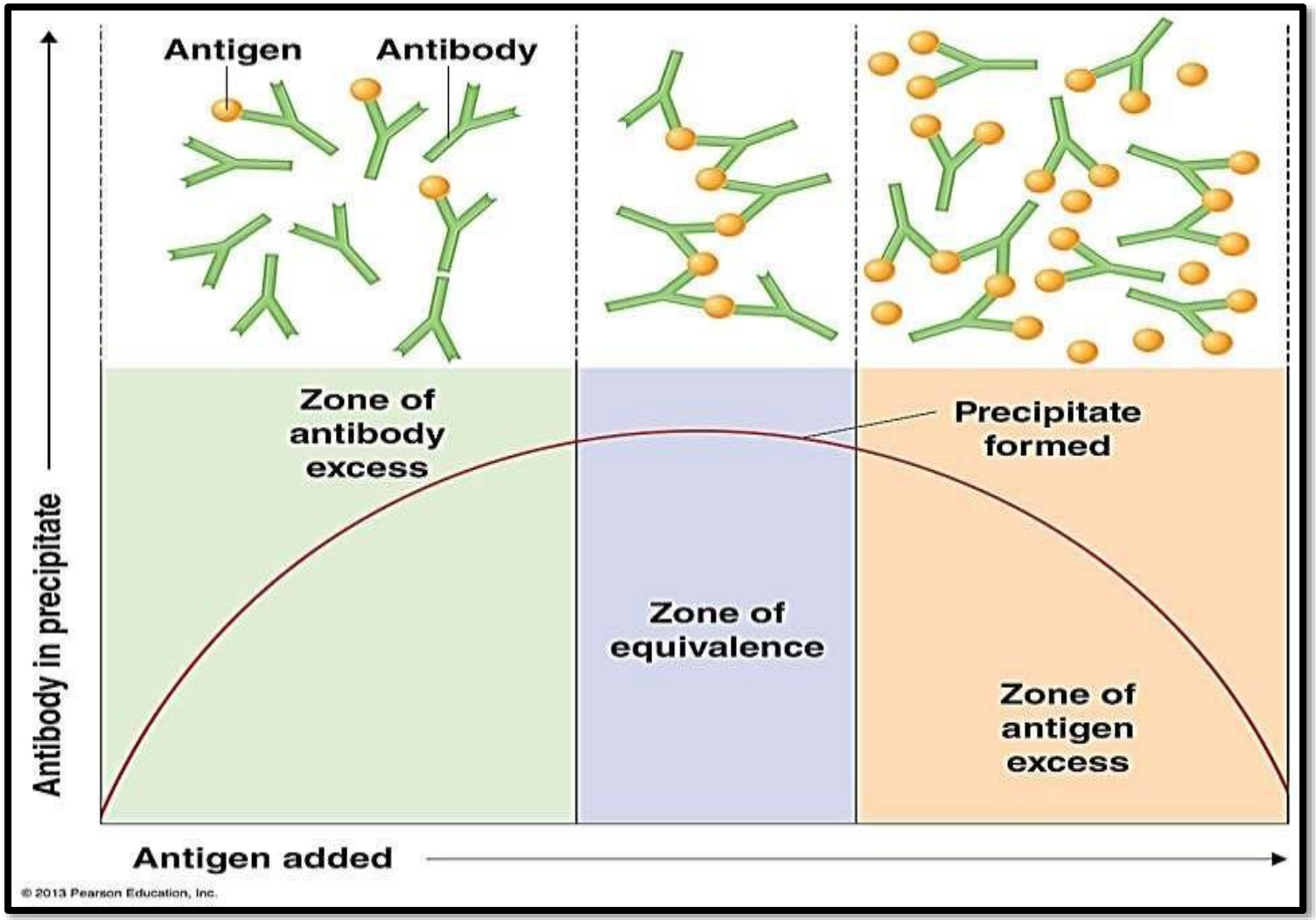
- Place constant amount of Ab in a series of tubes.

- Add increased amount of antigen.

- Antigen – Antibody reacts together resulting in precipitation.

- Plotting the amount of precipitate against increasing antigen conc. yields a 'precipitin curve'.

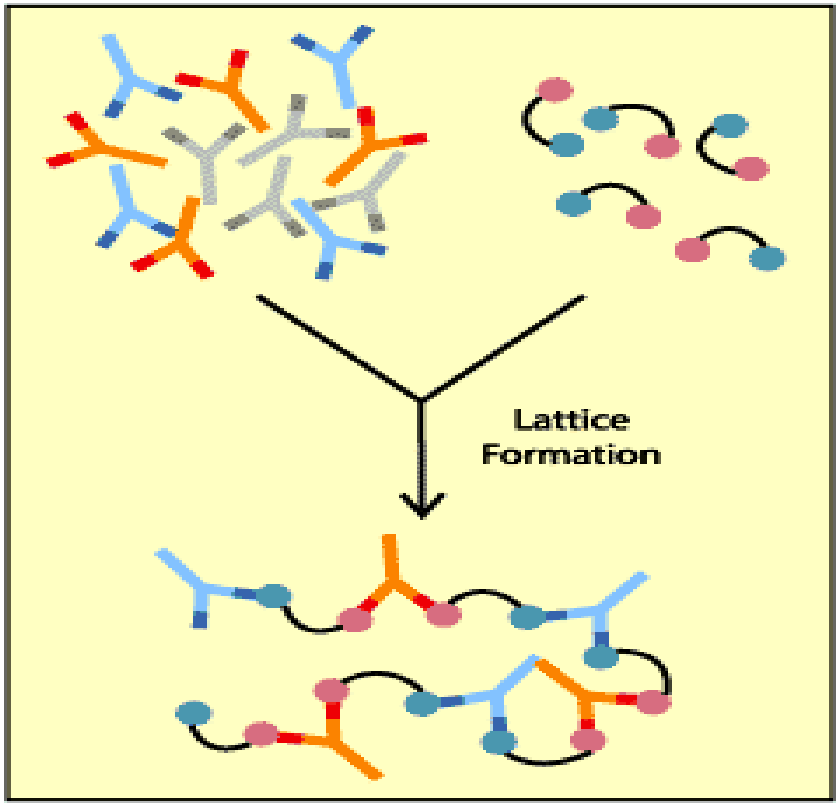
- Precipitation curve shows 3zones:
  - * Zone of Ab axis.
  - * Zone of equivalence.
  - * Zone of Ag axis.



PRINCIPLE

- Soluble antigen + antibody (in proper proportions) → visible precipitate

- Lattice formation (Ag binds with Fab sites of 2 Ab's)



2.Precipitation in Gels:

RADIAL IMMUNODIFFUSION:

-In these methods agar gel or similar gels are used on plates or petri-plates.

- Both Ag and Ab diffuse freely in the gel system in all directions.

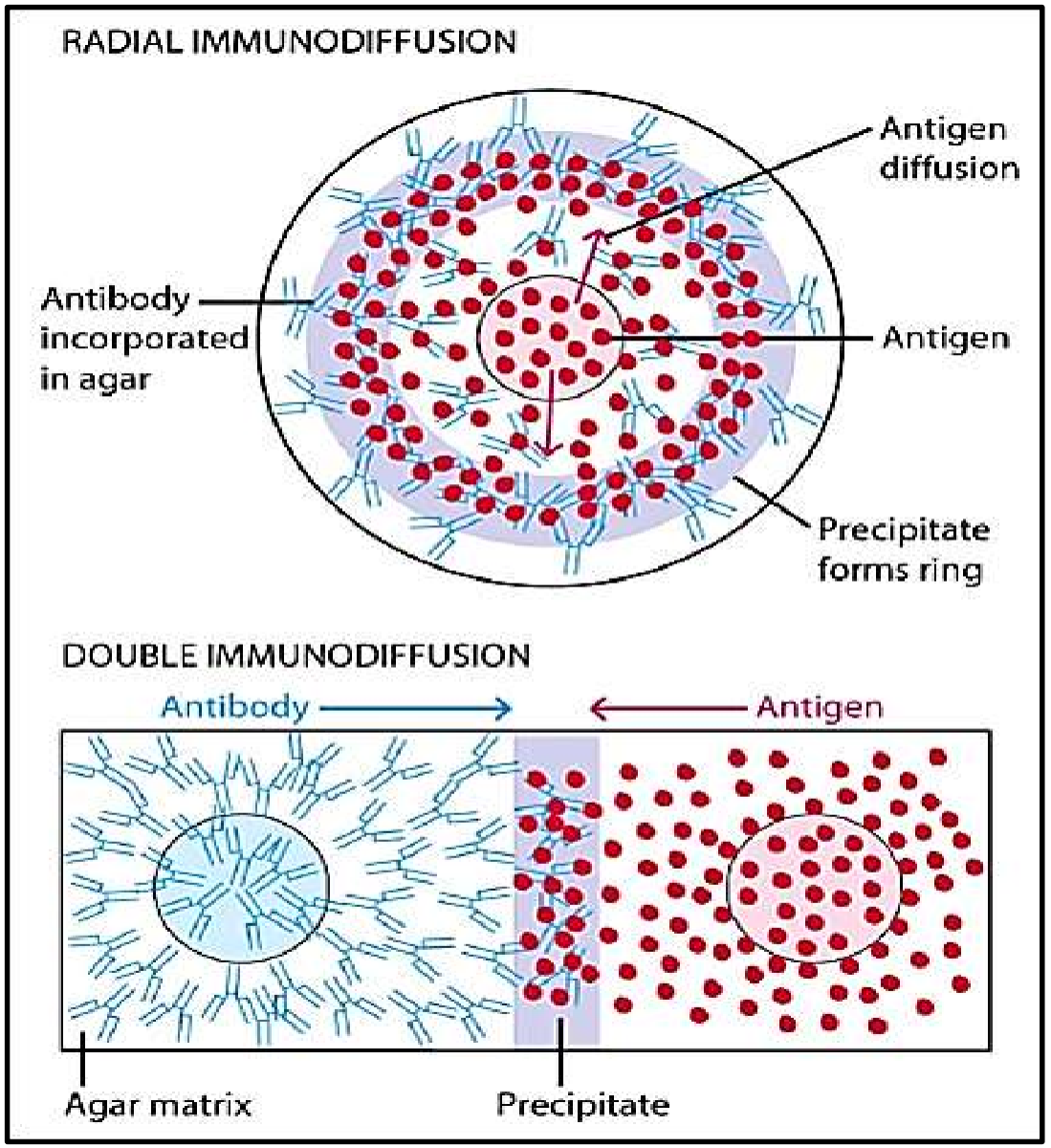
-At a certain point depending on the rate of diffusion & conc. of the reactants, a zone of equivalence will be formed, seen as a visible ppt.

- If Ag or Ab preparations are complex, multiple bands form.

- These are again of 2 types:

 * Single diffusion methods

* Double diffusion methods.



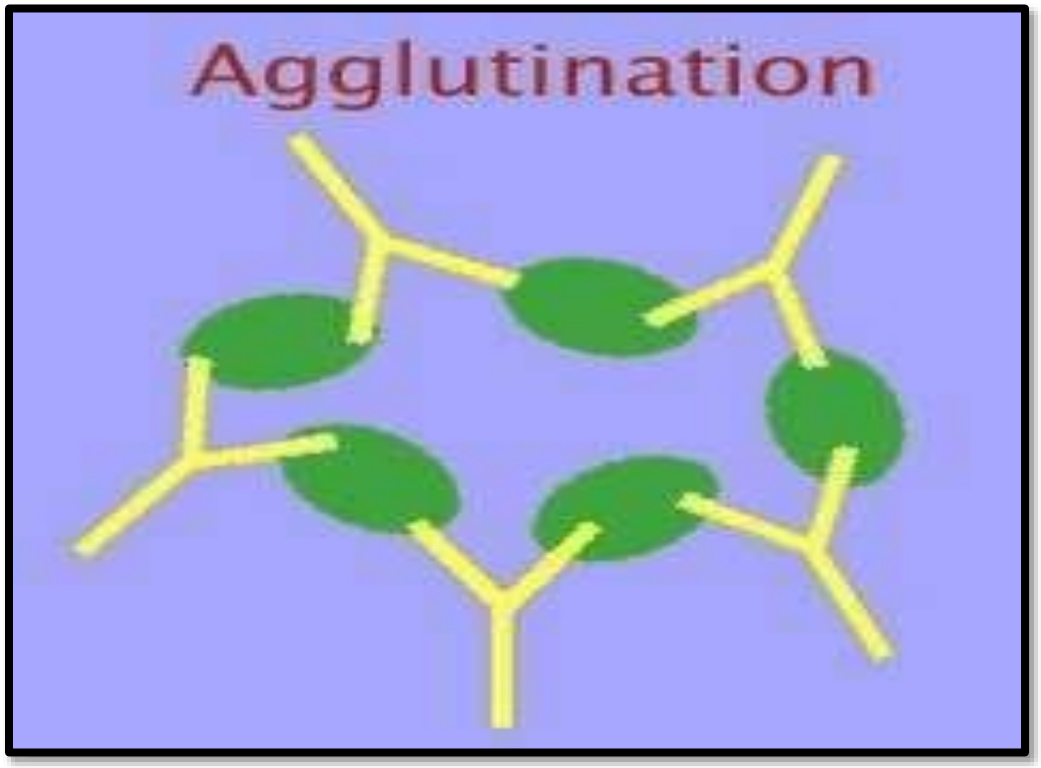
Precipitation reactions in gels

AGGLUTINATION

The interaction between antibody & particulate (Insoluble) antigen results in visible clumping called 'agglutination'.

-Antigens include:

- Bacteria
- White blood cells
- Red blood cells
- Latex particles



-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'.

-Agglutination can be performed in a tube or on a glass slide e.g. ABO blood grouping.

-Ab is divalent and cross links the multivalent antigen to form clumps.

TUBE AGGLUTINATION:

- Serum containing Ab is diluted serially with saline in small test tubes, a constant volume of Ag suspension is added.

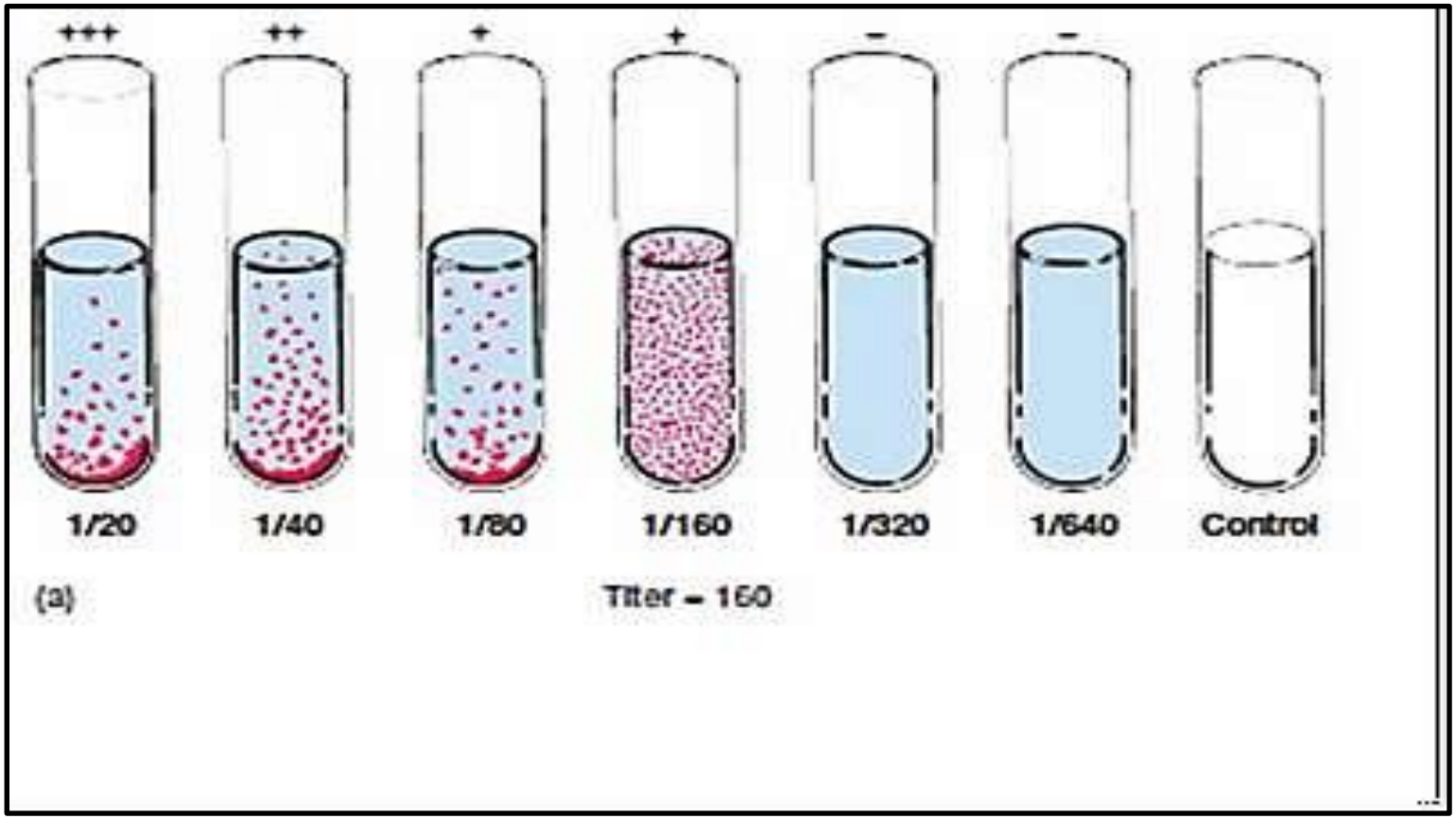
- Control tube is kept which has no antiserum.

- The tubes are incubated until visible agglutination is observed.

-The tube showing highest agglutination is referred to as the 'titre'.

APPLICATION -> Widal test is used for the estimation of typhoid fever

In this test Ab content of the patient's serum, is measured by adding a constant amount of antigen (Salmonella typhi) to the serially diluted serum.



PASSIVE AGGLUTINATION:

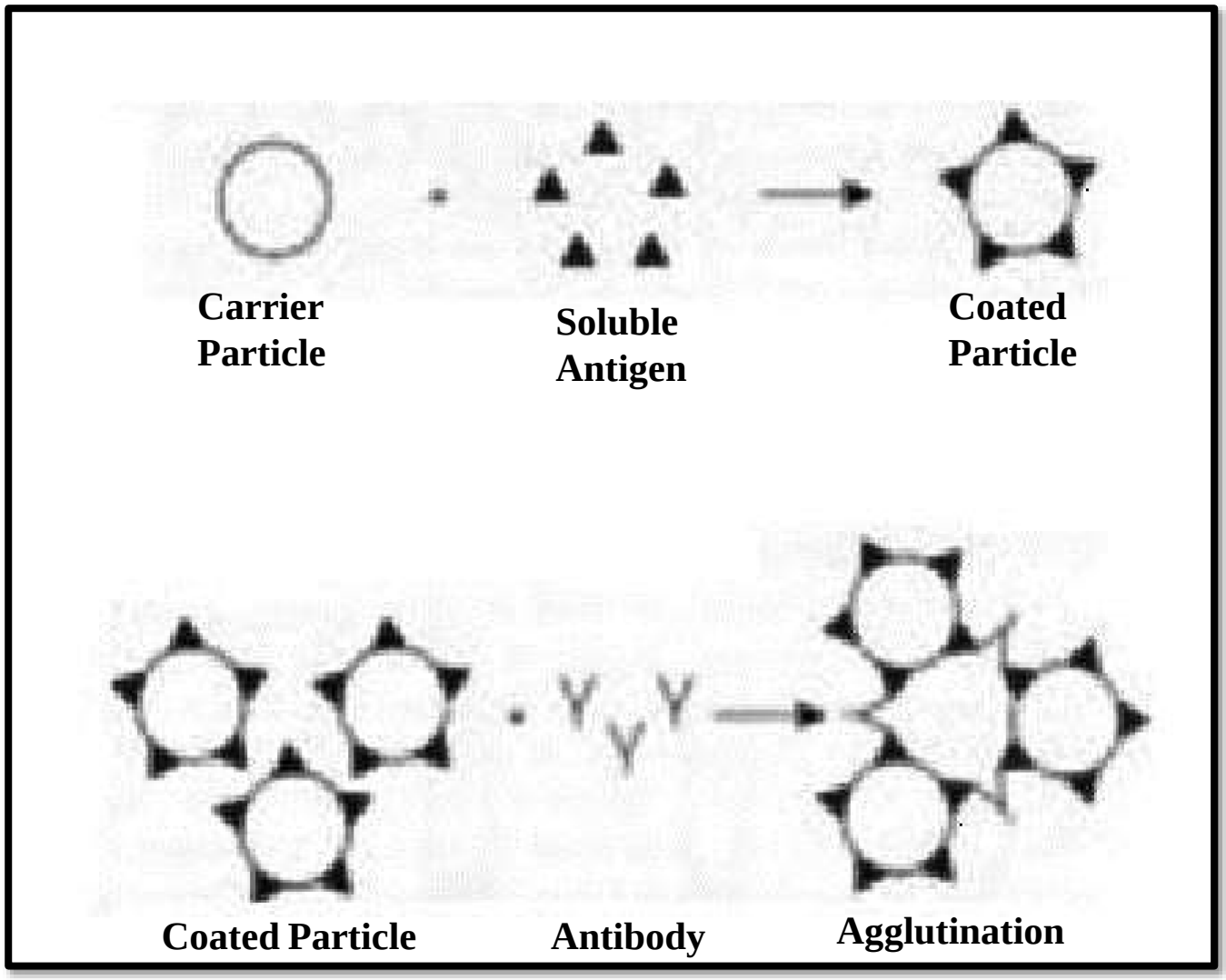
- Ag is coated on the surface of a carrier particle.

-This helps to convert a precipitation reaction to an agglutination reaction making the reaction more sensitive.

- The carrier particles used can be RBC, latex particles or bentonite.

-When patients serum is mixed with these, it leads to agglutination.

APPLICATION -> diagnosis of Rheumatoid arthritis.

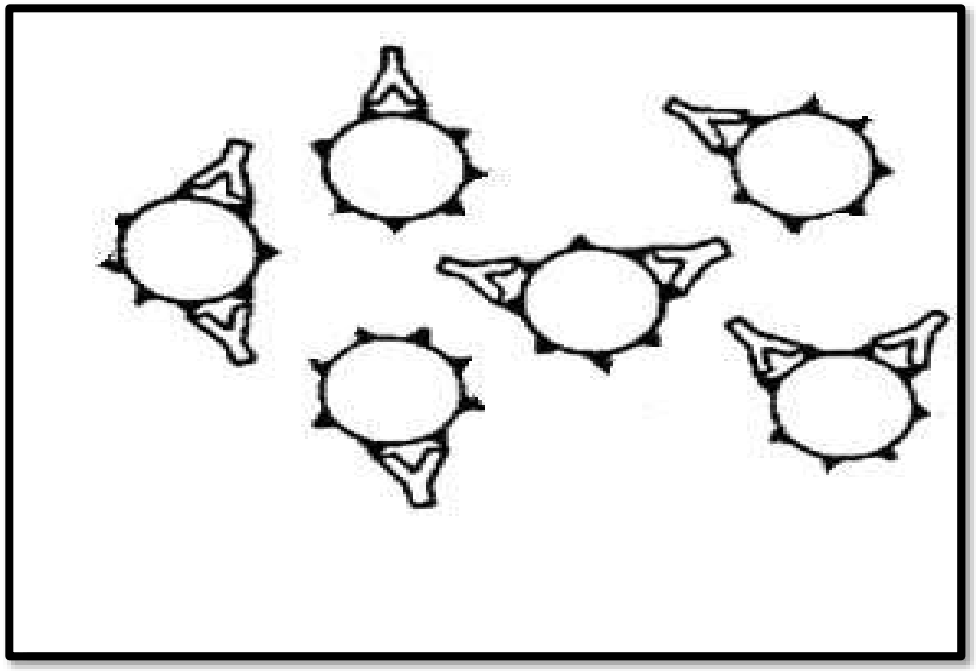

Carrier Particle
Soluble Antigen
Coated Particle
Coated Particle
Antibody
Agglutination

PHASES OF AGGLUTINATION

1. PRIMARY PHASE (SENSITIZATION)

Ab reacts with a single epitope on the surface of Ag.

STAGE 1->

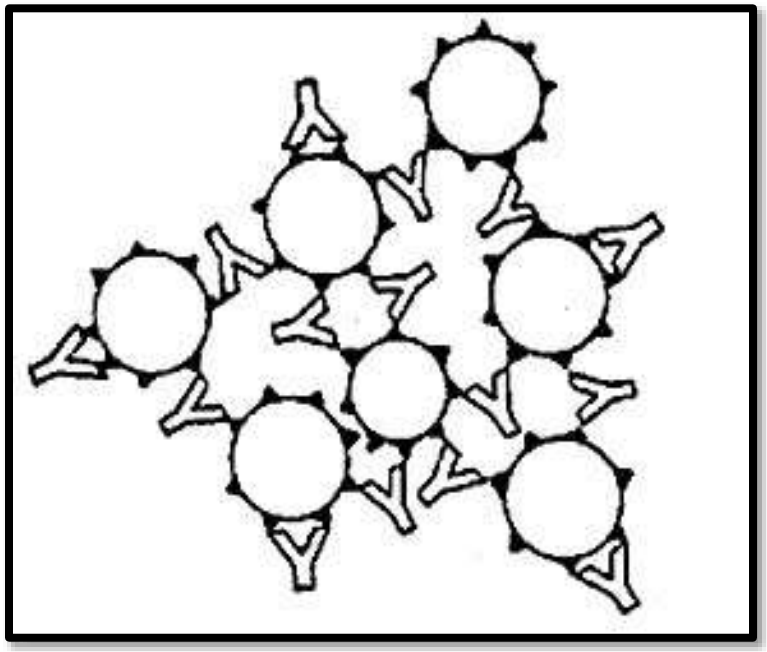


-Ab molecules attach to their corresponding Antigenic site (epitope) on membrane. There is no visible clumping.

2. SECONDARY PHASE (LATTICE FORMATION)

Ab bridges gap so one Fab portion is attached to an epitope on each of 2 adjacent particles (dependent on environmental conditions & the relative conc. of Ag & Ab)

STAGE 2 ->



- Ab molecules crosslink RBCs forming a lattice that results in visible clumping or agglutination

FACTORS

- Elevation or decrease of temperature.
- decreasing the temperature from 37°C to 4°C slows the rate of reaction 20-fold

- Motion (shaking, stirring, centrifugation).

- pH.

Most antibodies are not affected by changes in pH within the **range 5.5-8.5**. ... Below pH 4 and above pH 9, antigen-antibody complexes are largely dissociated

- Class of antibody (IgM/IgG).

HEMAGGLUTINATION TEST

- Type of agglutination test performed on RBCs.

- It has two types:

1. **Active:**

i) The antigen is the RBC itself.

ii) Viruses can clump red blood cells from one species or another (active hemagglutination)

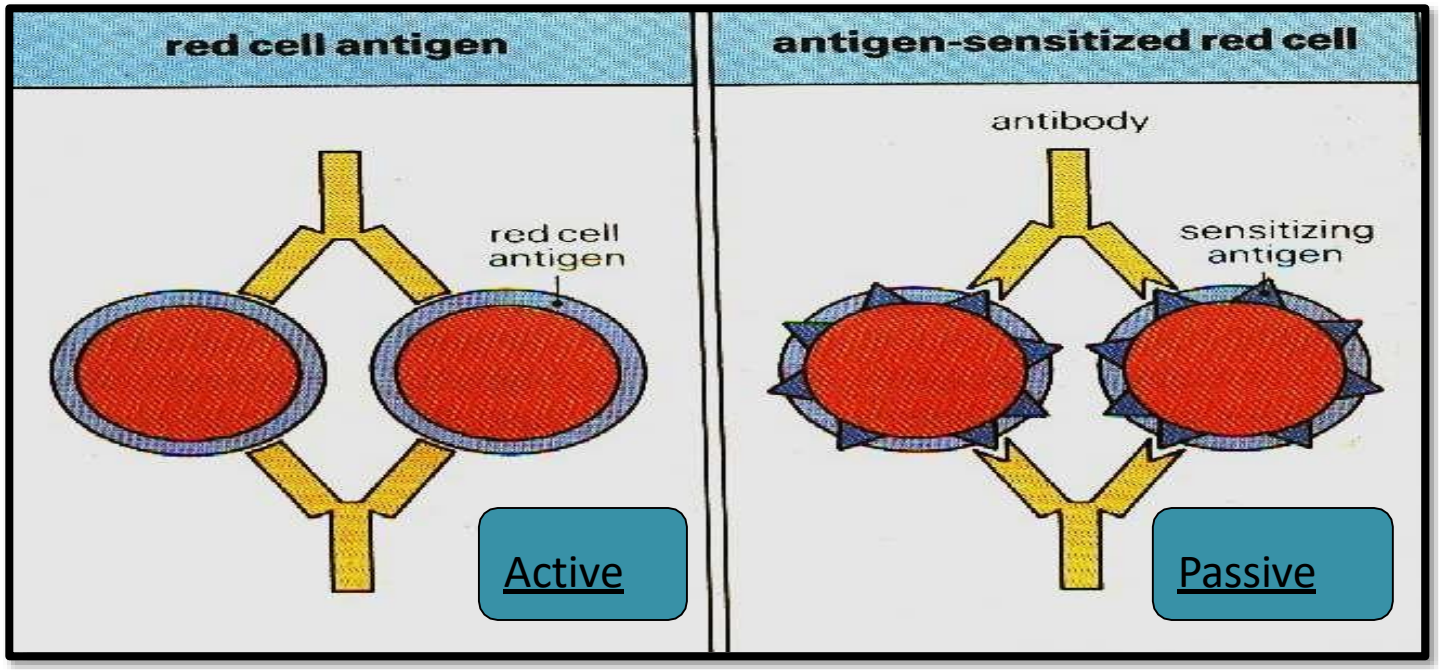
Example is the test used in ABO grouping.

2. Passive:

i) The antigen here is not the RBC.

ii) The RBC absorbs it and expresses it on the surface.

iii) It will form clumps when mixed with antibodies i.e. red cells are passive carriers .



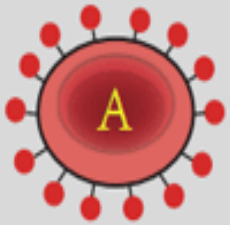
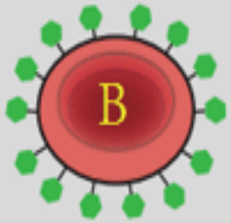
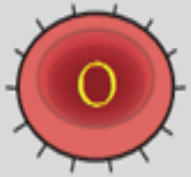
APPLICATIONS:

- Blood typing.
- Bacterial infections

LIMITATIONS:

- Time consuming (1 day)
- Cannot distinguish IgG from IgM.

BLOOD Group System

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	 A antigen	 B antigen	 A & B antigens	None


THANK
YOU!