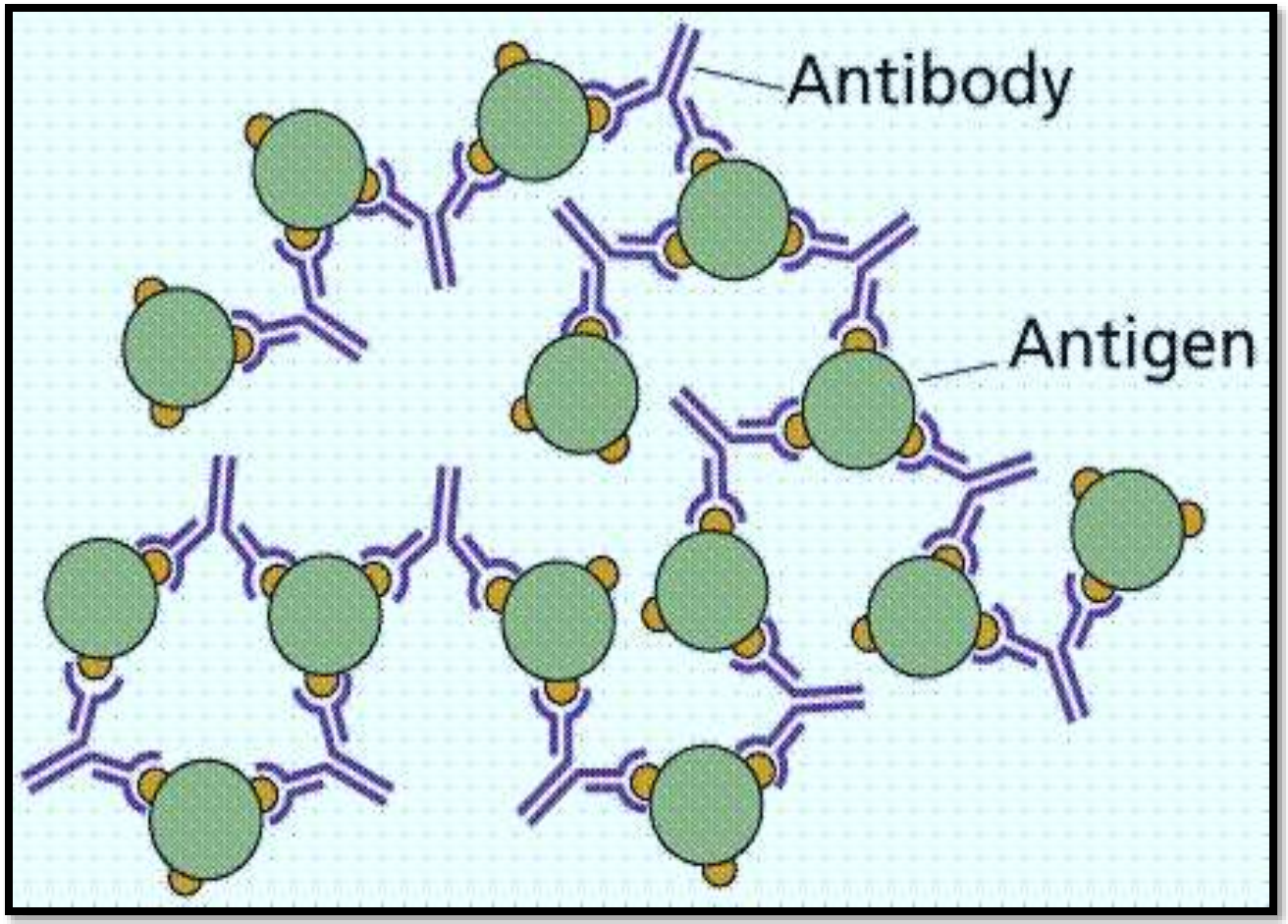
ANTIGEN & ANTIBODY REACTIONS



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 ‘serological reactions’.

- 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.

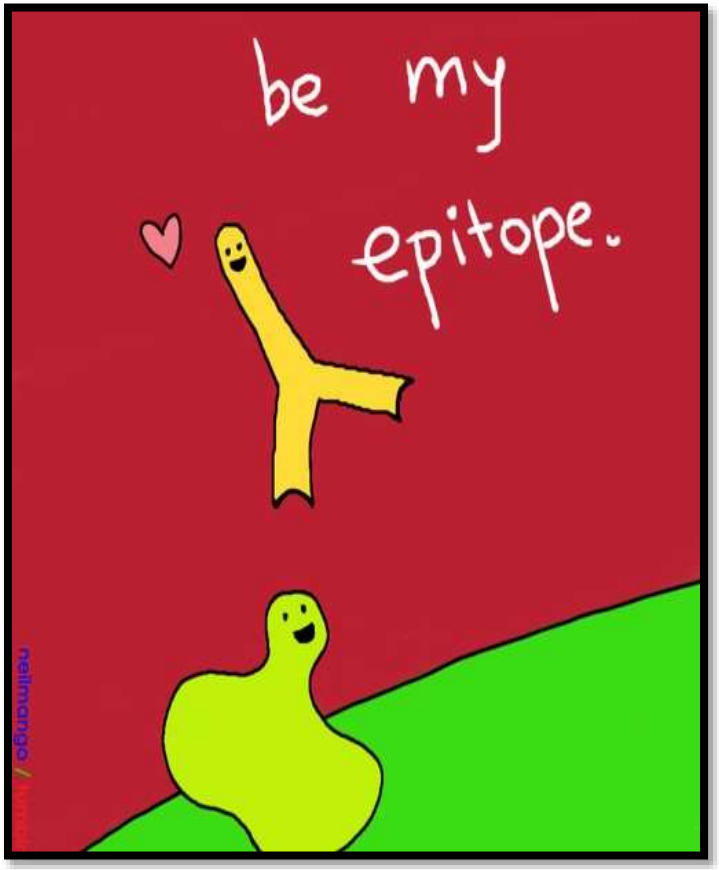
SALIENT FEATURES

- Specificity.

- Immune complex.

- Binding Site of Antigen.

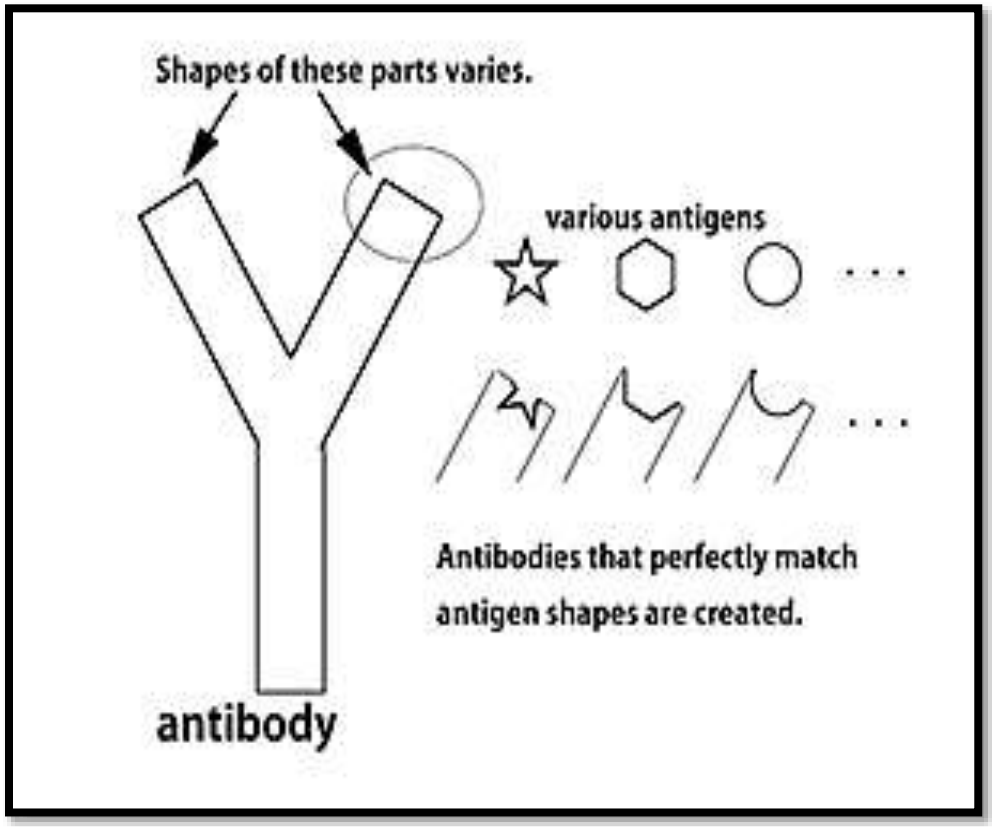
- Binding Force of Antigen.

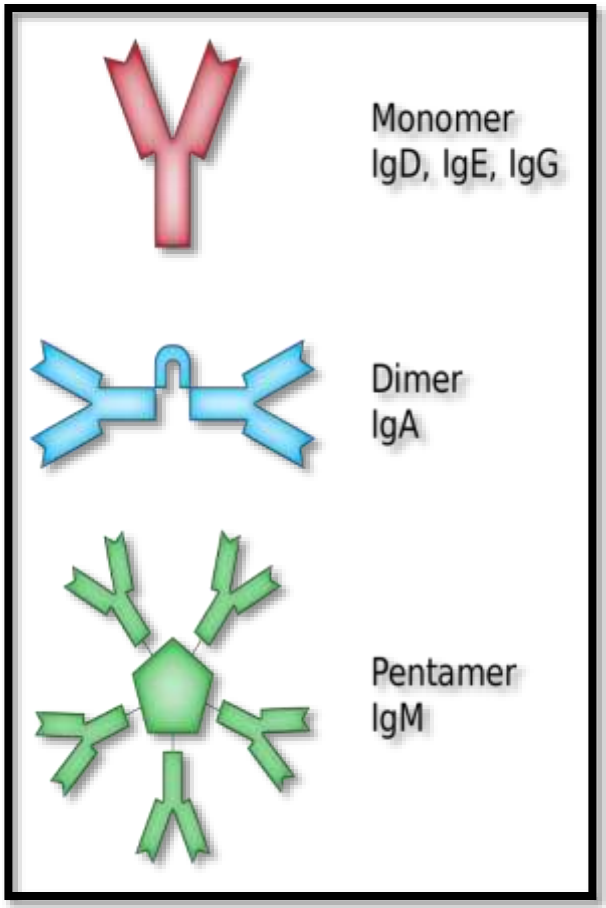


1. SPECIFICITY:

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.

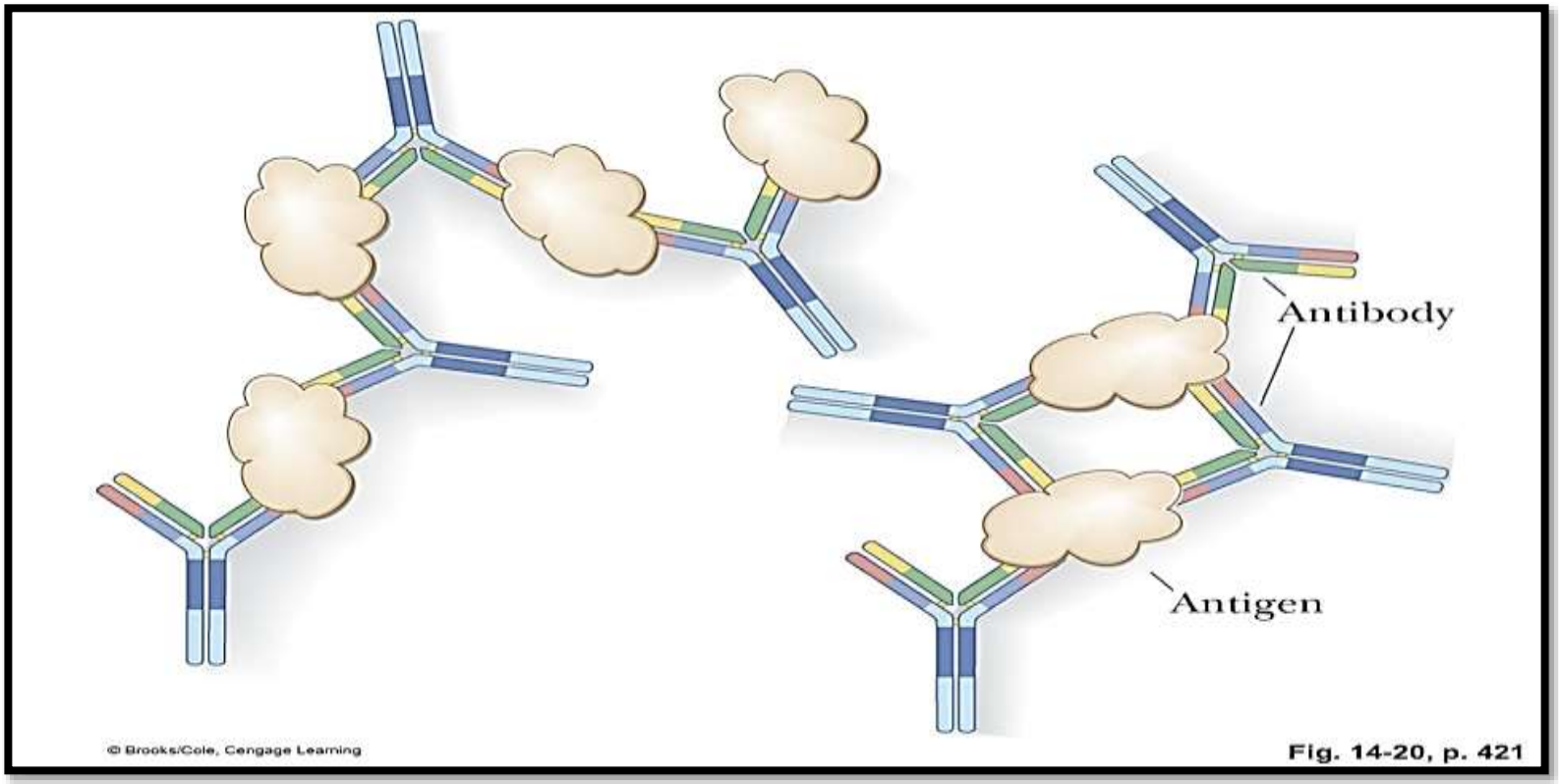




2. IMMUNE COMPLEX:

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

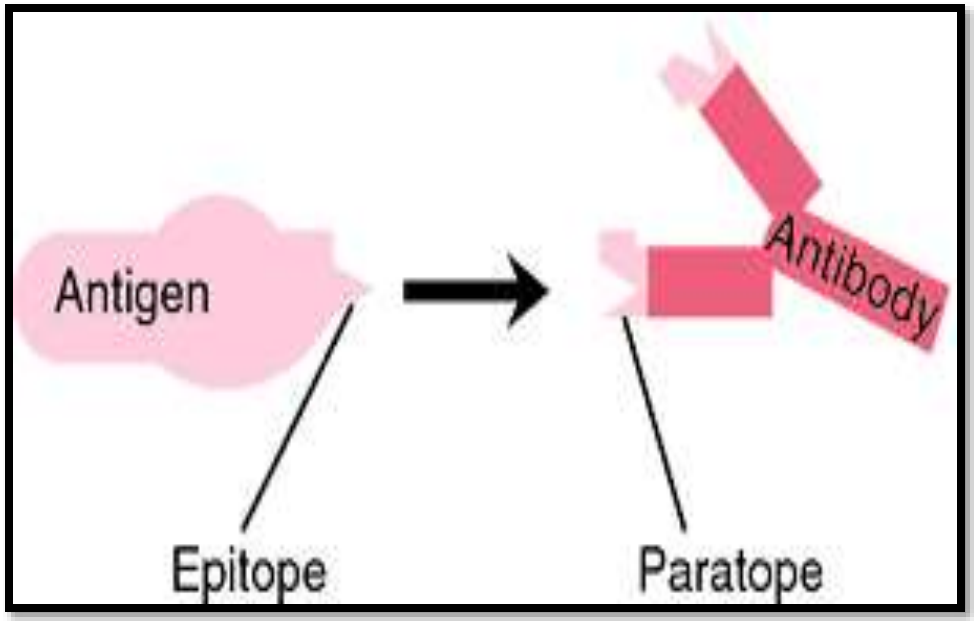
Ag + Ab → Ag-Ab complex

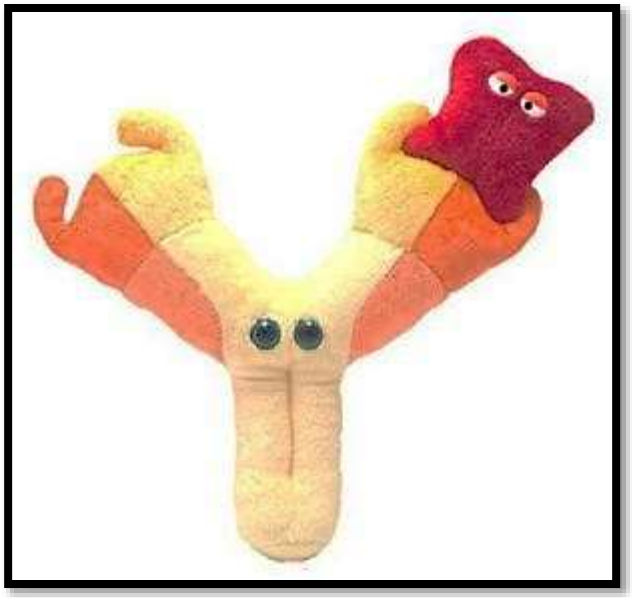


3. BINDING SITE OF Ag:

- 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:

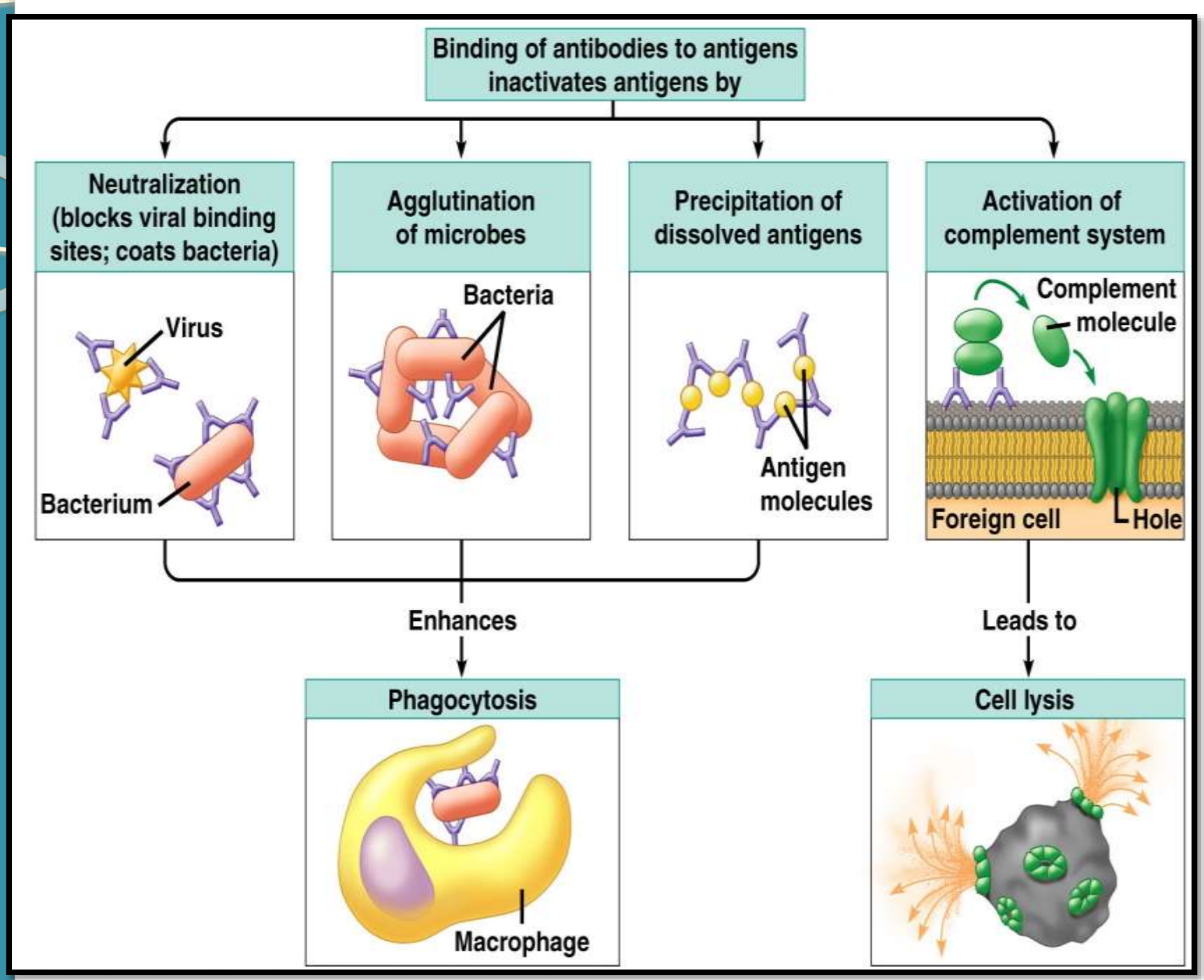
- Precipitation Reaction.

- 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 occurs in two media:
    * Liquid
    * Gel

1. Precipitation in Liquids:

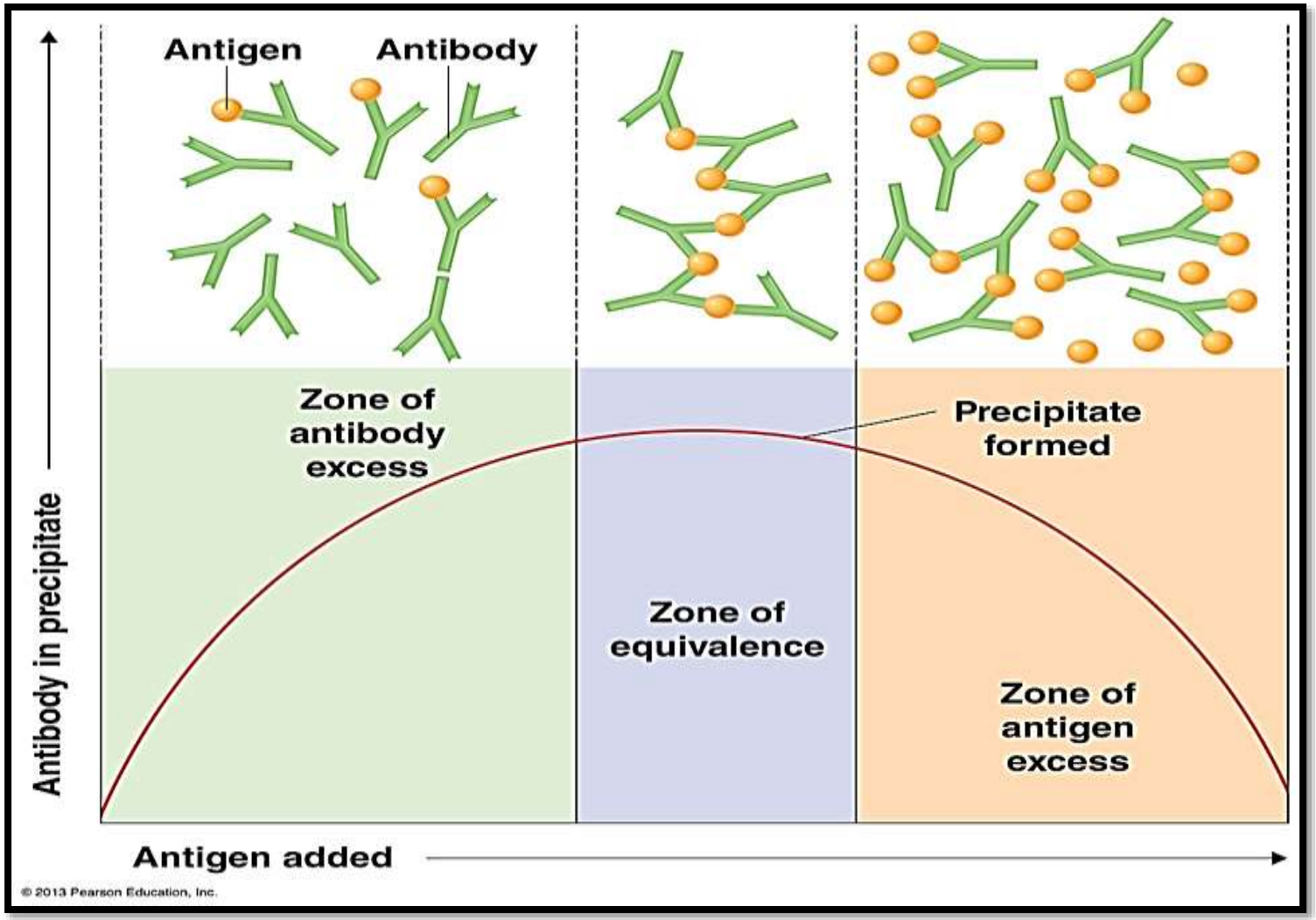
- Place constant amount of Ab in a series of tubes.

- 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'.

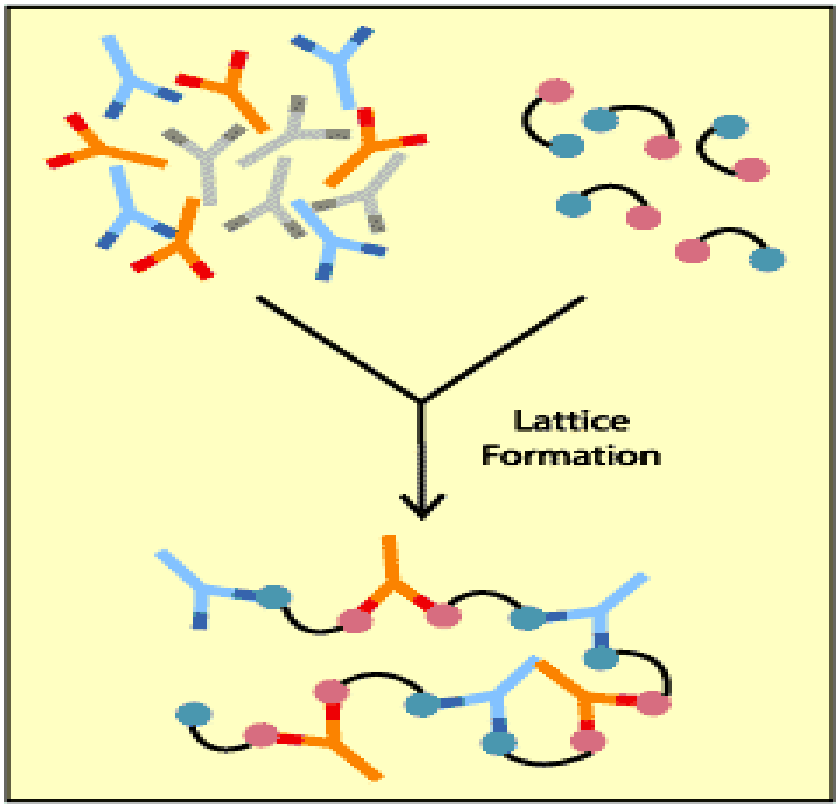
- Precipitation curve shows 3 zones:
    * Zone of Ab axis.
    * Zone of equivalence.
    * Zone of Ag axis.



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.

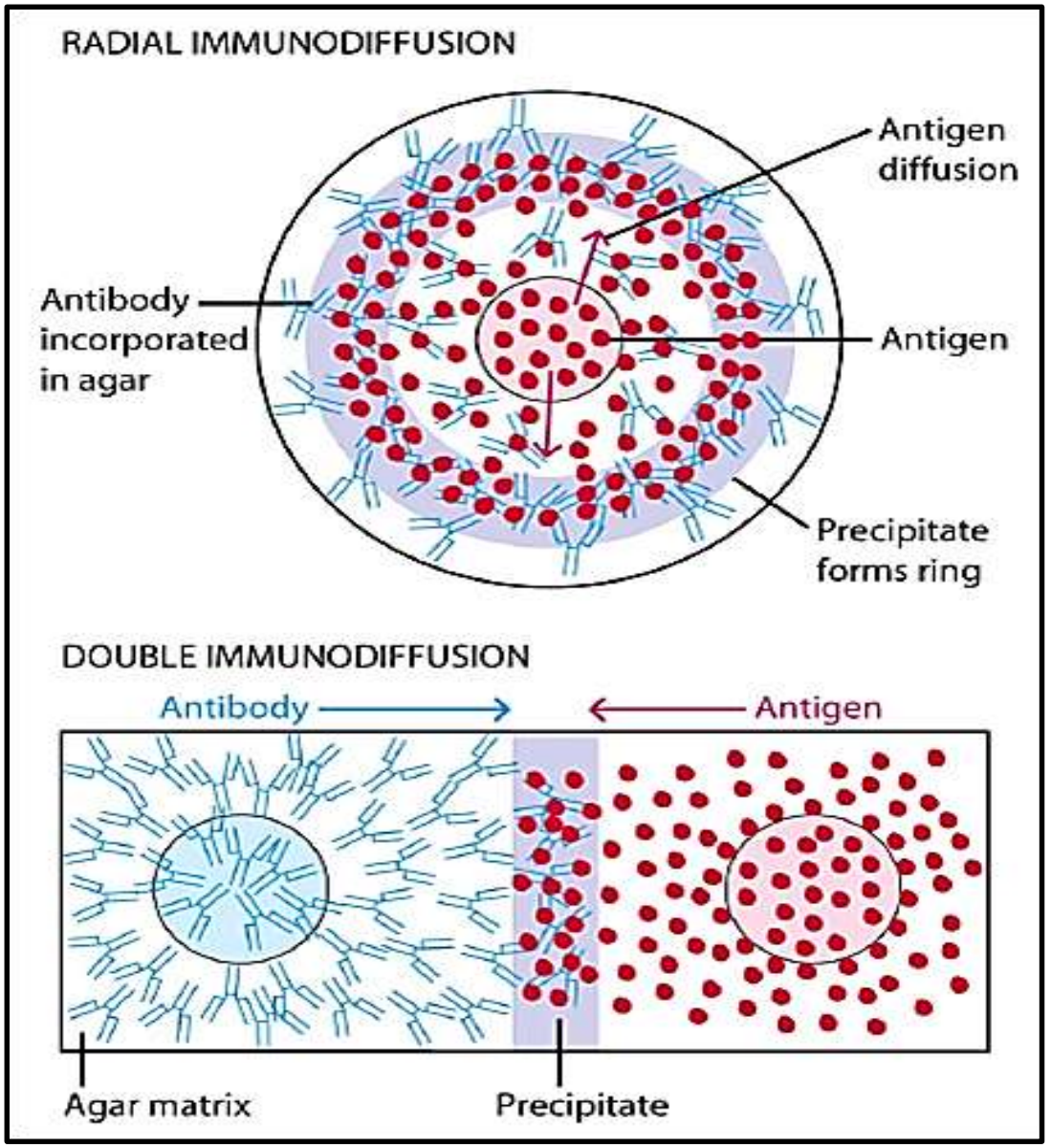
- At a certain point depending on the rate of diffusion & conc. of the reactants, a zone of equivalence will be formed, seen as a visible ppt.

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

- These are again of 2 types:

 * Single diffusion methods

* Double diffusion methods.

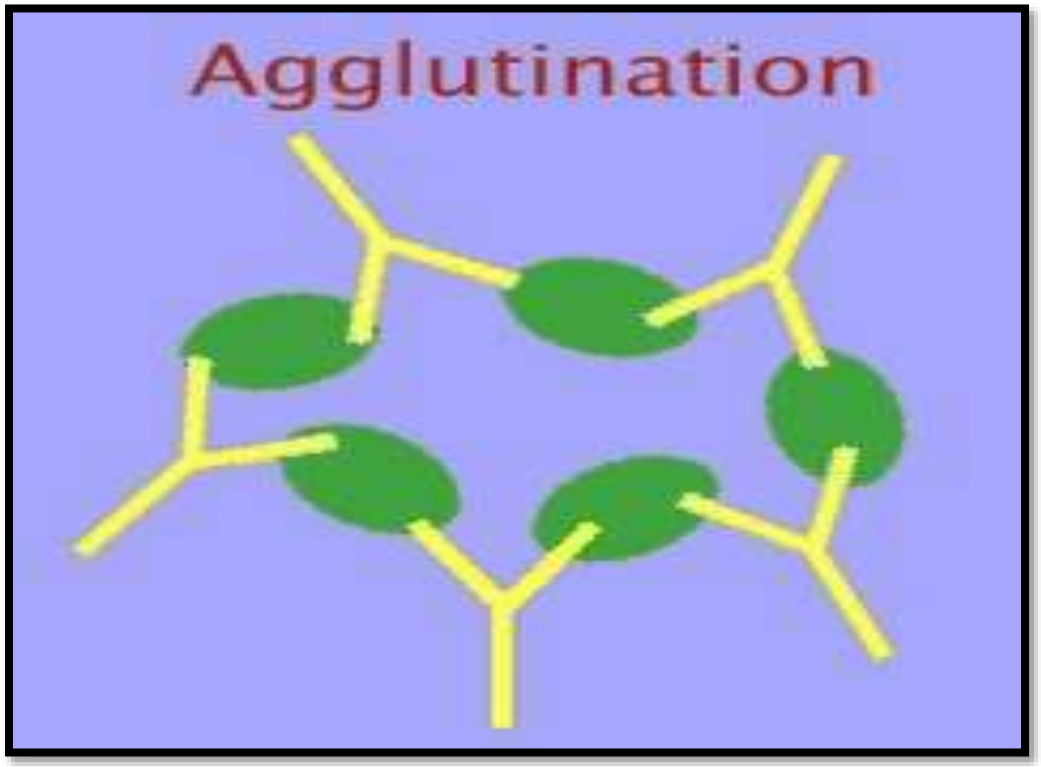


Precipitation reactions in gels

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.

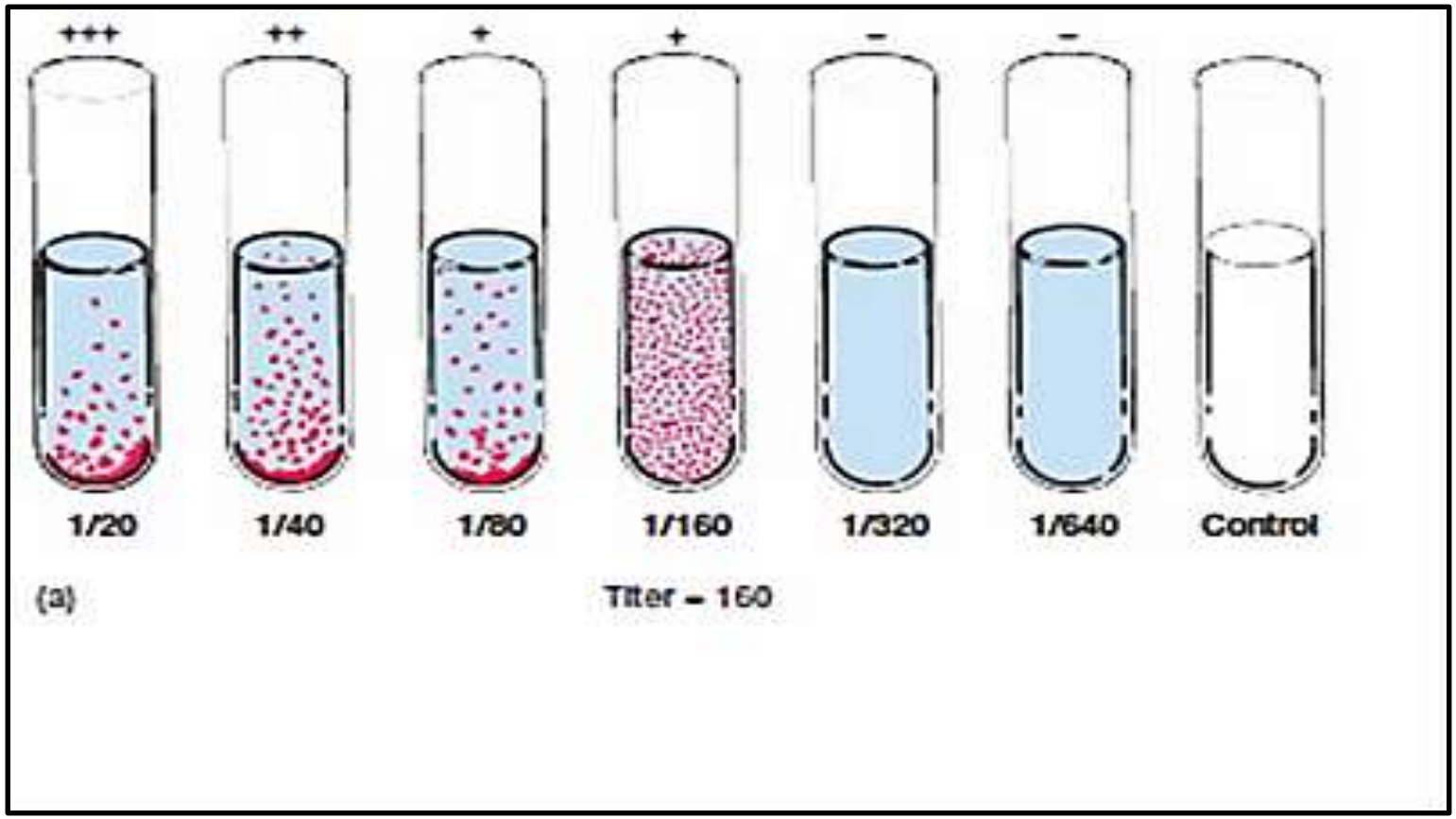
- Control tube is kept which has no antiserum.

- 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:

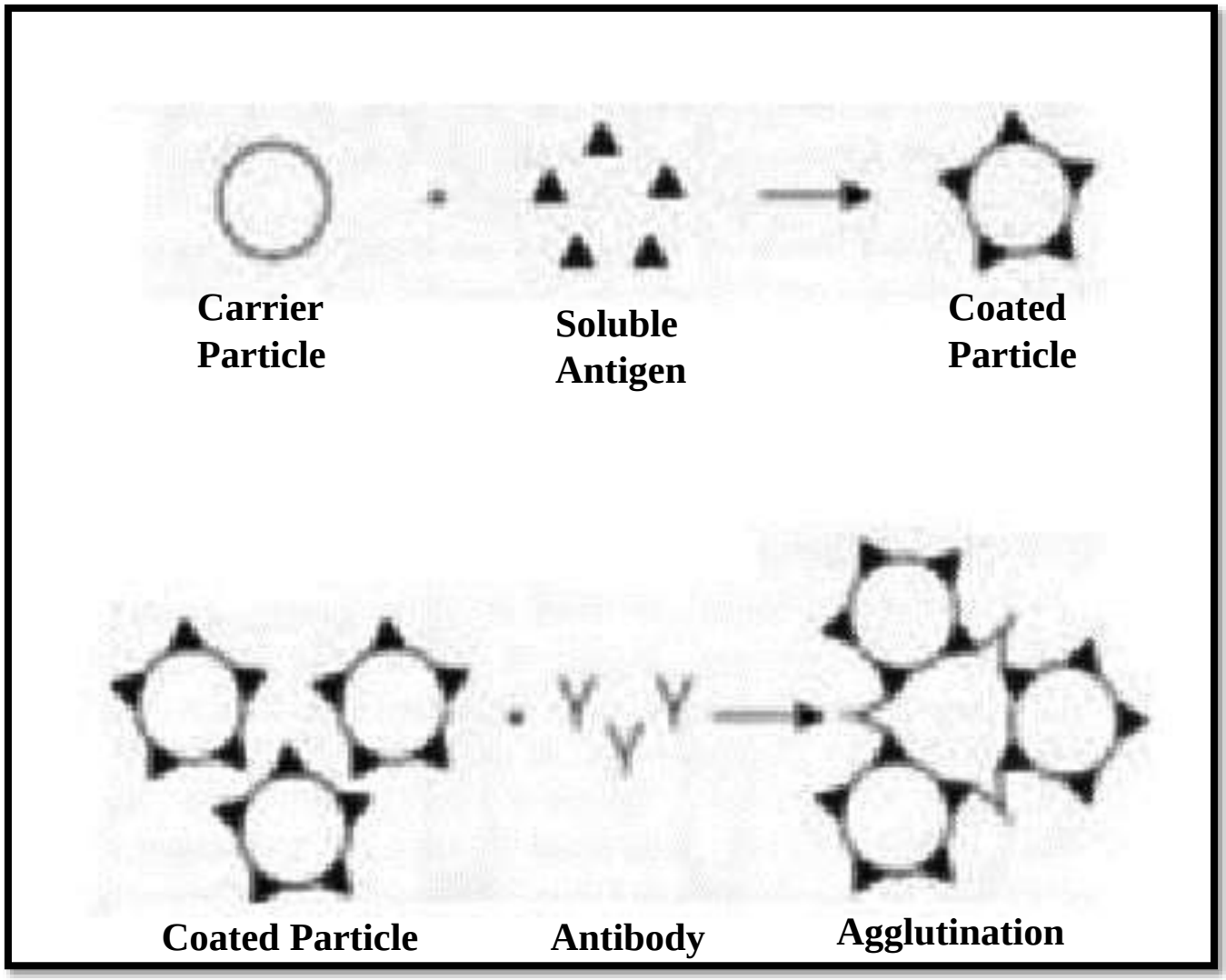
- Ag is coated on the surface of a carrier particle.

- 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.

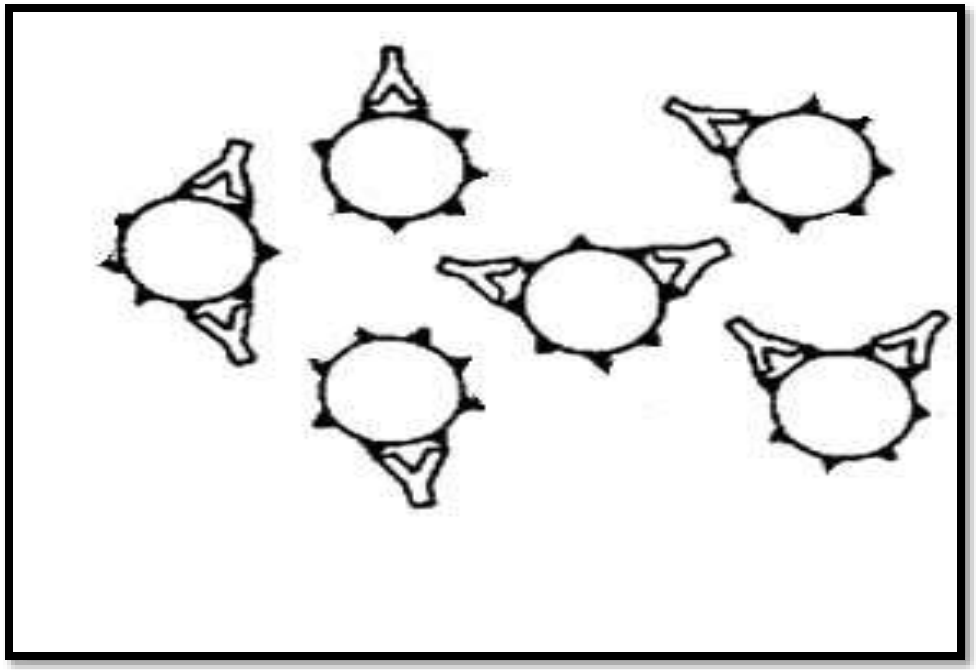

Carrier Particle
Soluble Antigen
Coated Particle
Coated Particle
Antibody
Agglutination

PHASES OF AGGLUTINATION

1. PRIMARY PHASE (SENSITIZATION)

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

STAGE 1 ->

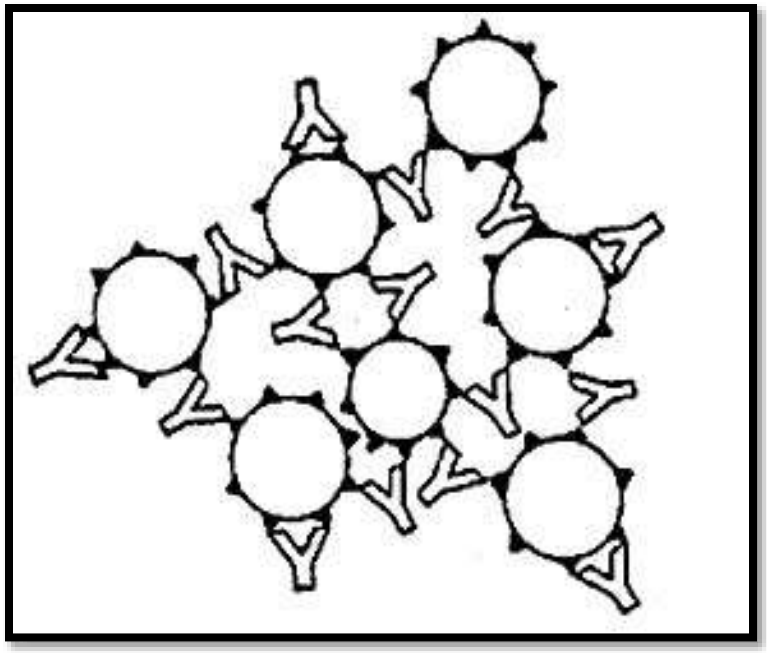


- Ab molecules attach to their corresponding Antigenic site (epitope) on membrane. There is no visible clumping.

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

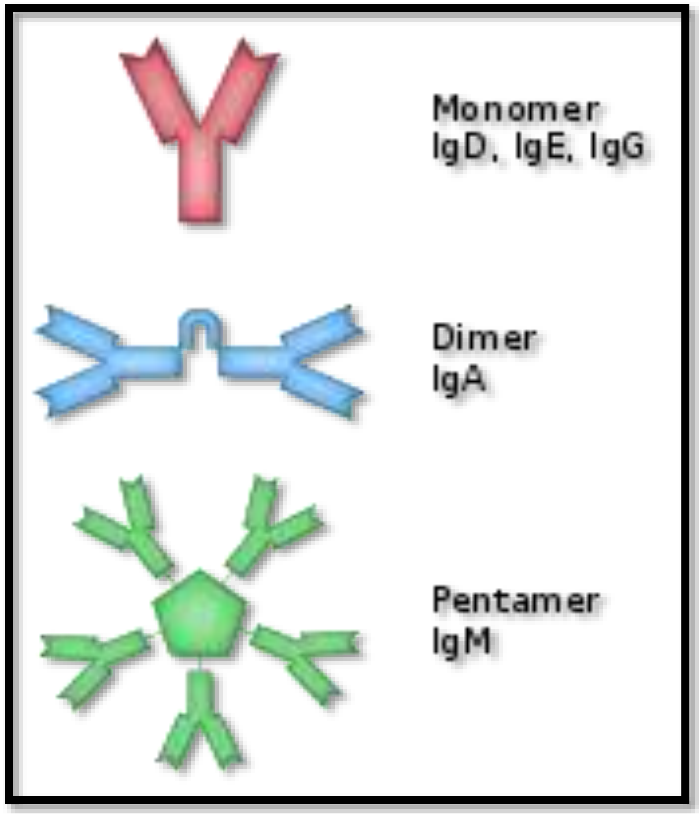
FACTORS

- Elevation or decrease of temperature.

- Motion (shaking, stirring, centrifugation).

- pH.

- 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.

Agglutination

The ABO Blood System

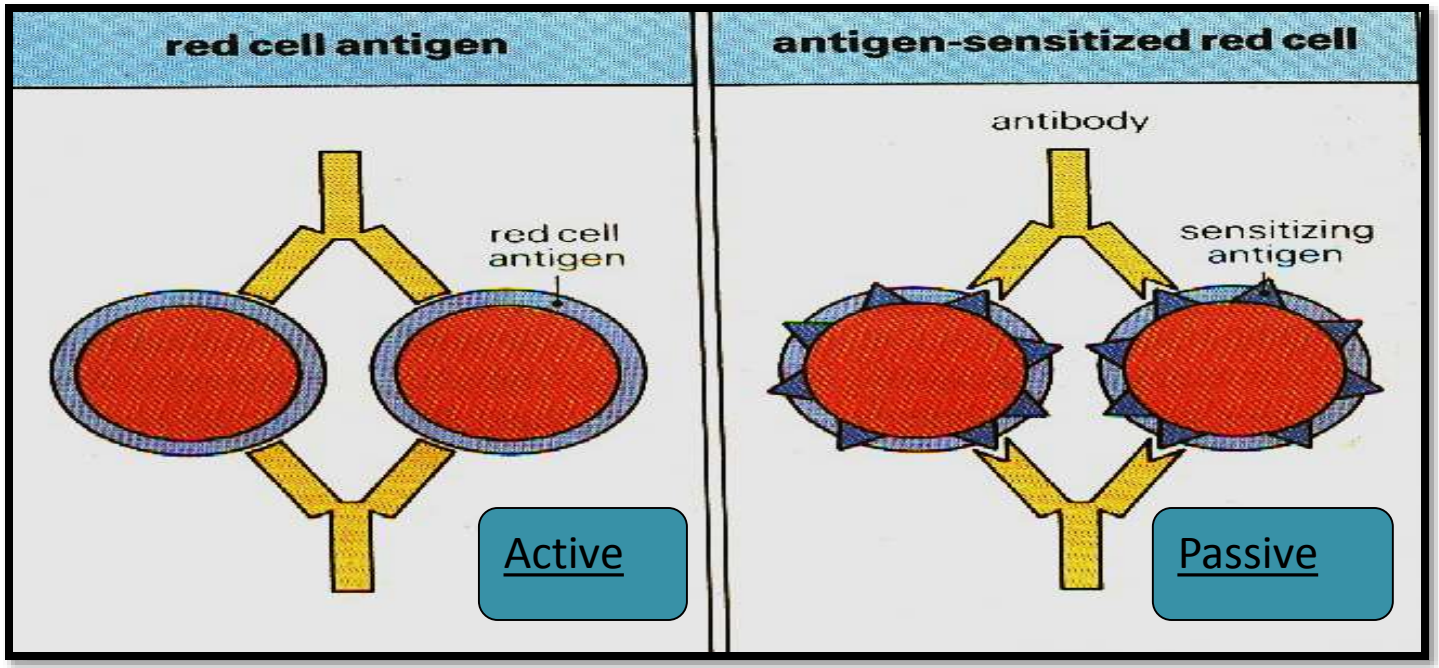
Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin

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:

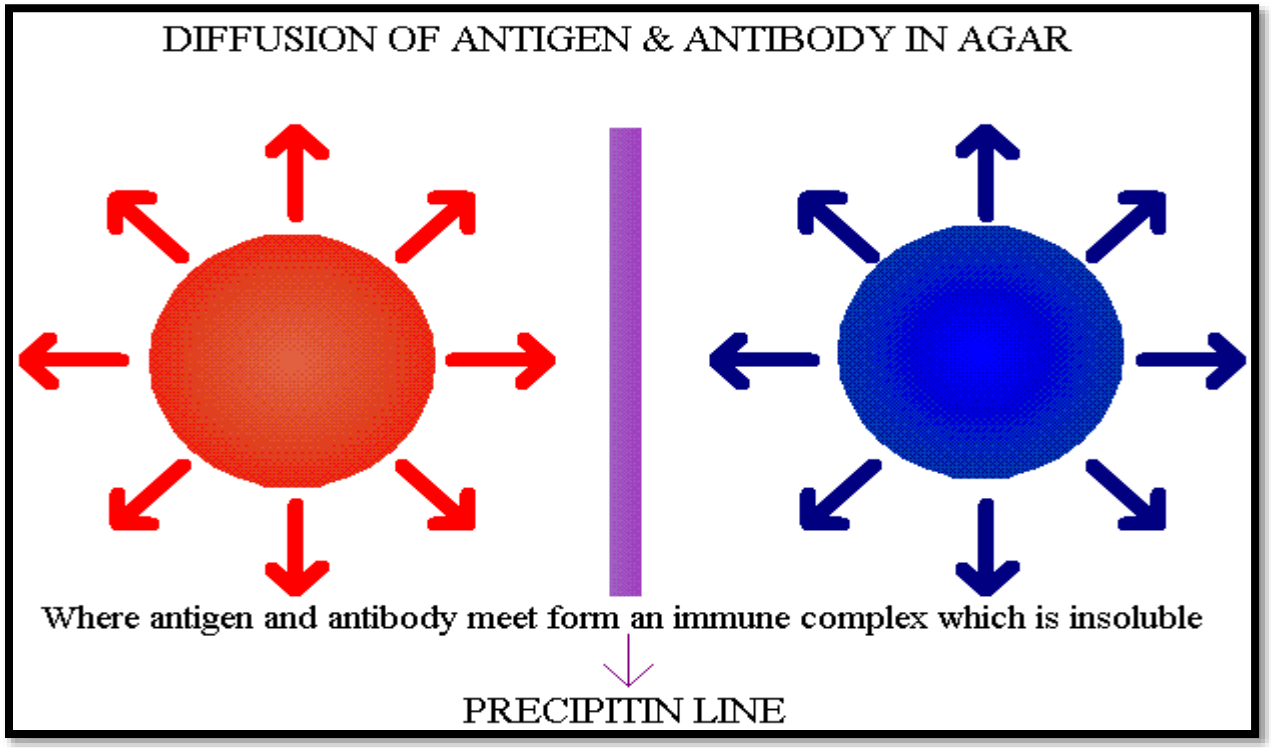
- Blood typing.
- Bacterial infections

LIMITATIONS:

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

IMMUNODIFFFUSION

A technique for studying reactions between antigens & antibodies by observing precipitates formed by the combination of specific antigens & antibodies diffused in gel in which they have been separately placed.



ADVANTAGES:

a) Precipitin band is visible which can be stained for preservation.

b) It can be used to detect identity, cross-reaction & non-identity b/w antigens in a mixture.

TYPES OF IMMUNODIFFUSION

They are classified on the basis of:

a) Number of reactants diffusing

b) Direction of diffusion

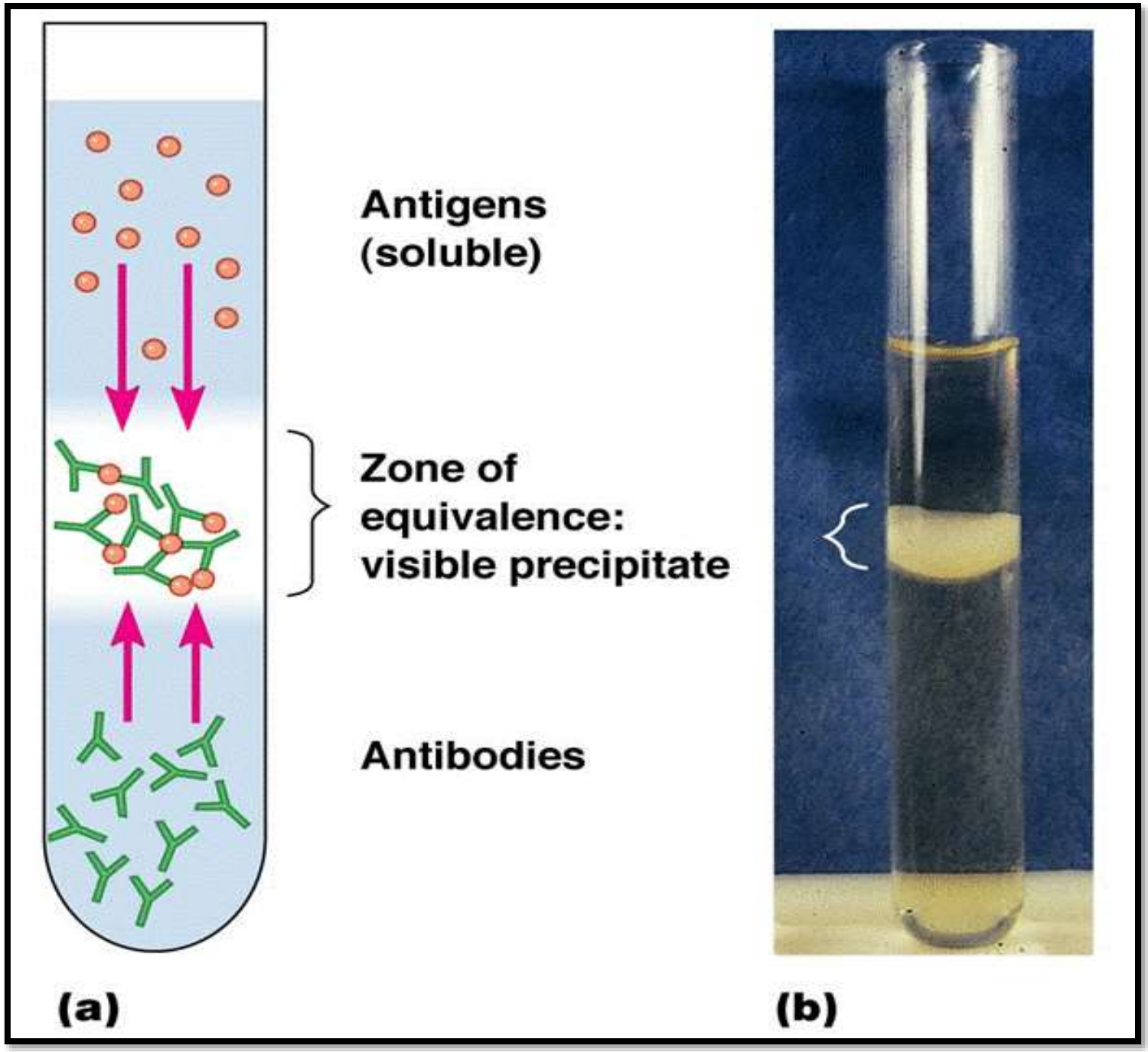
1. SINGLE DIFFUSION IN ONE DIRECTION

As the name suggests it is the single diffusion of antigen in agar in one direction.

This technique was pioneered by 'Oudin' who first time used gels for precipitation.

Procedure:

1. Ab is added in agar gel in test tube
2. Ag solution is poured over it
3. Ag diffuses downward towards Ab
4. Line of precipitation is formed
5. The number of bands shows number of Ag present in solution

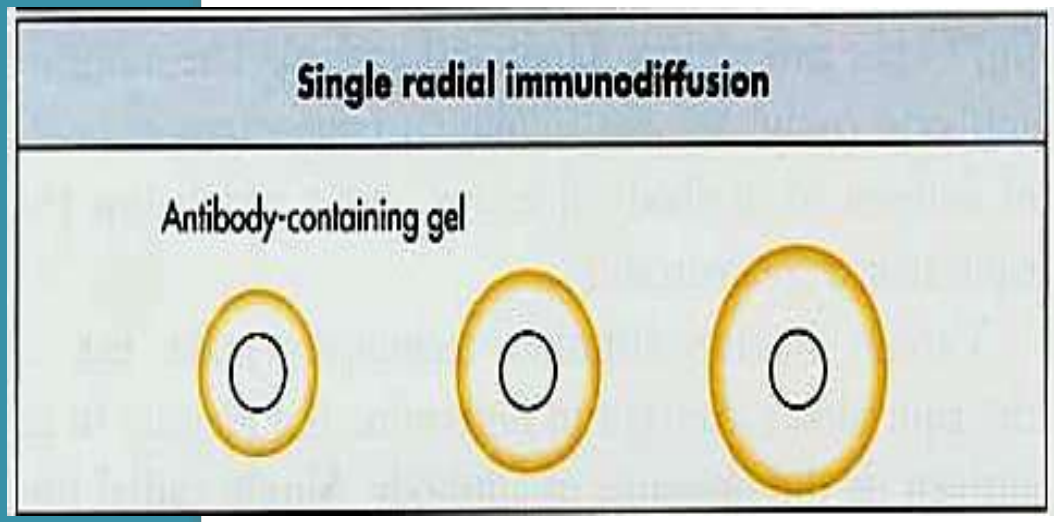

Antigens
(soluble)
Zone of
equivalence:
visible precipitate
Antibodies
(a)
(b)

2. SINGLE DIFFUSION IN TWO DIMENSIONS

Radial Immunodiffusion

Single diffusion in 2 dimensions is also called 'radial immunodiffusion'.

It is used in immunology to detect quantity of Ag by measuring the radius surrounding samples of the Ag, marking the boundary b/w it & A



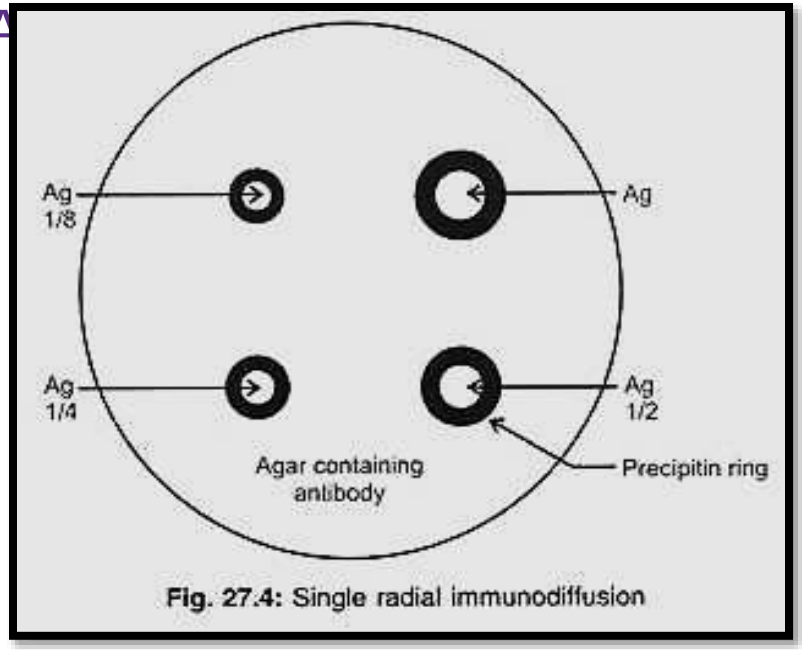
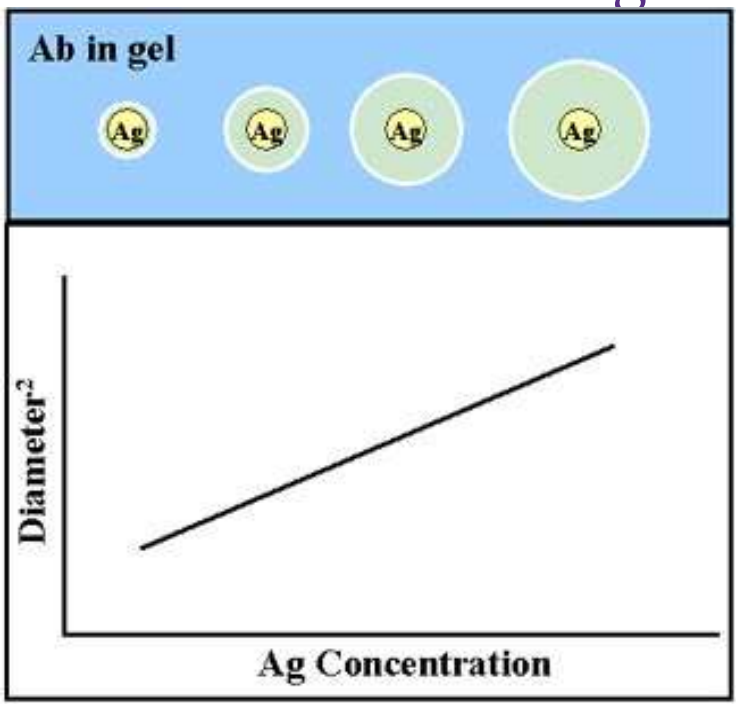
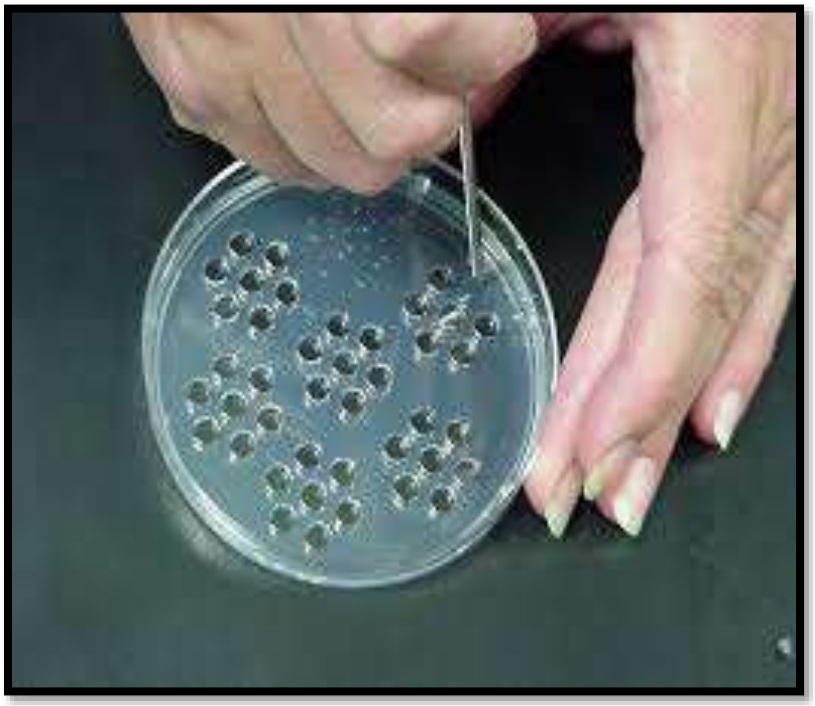


Fig. 27.4: Single radial immunodiffusion

Procedure:

1. Anti-sera sol containing Ab in agar sol is placed on a slide/petri dish.
2. Ag is added to the wells cut on the surface of the gel
3. Ab present in the gel reacts with Ag which diffuses radially from well & forms a ring shaped band of precipitation
4. Diameter of the ring is directly proportional to the concentration of the Ag



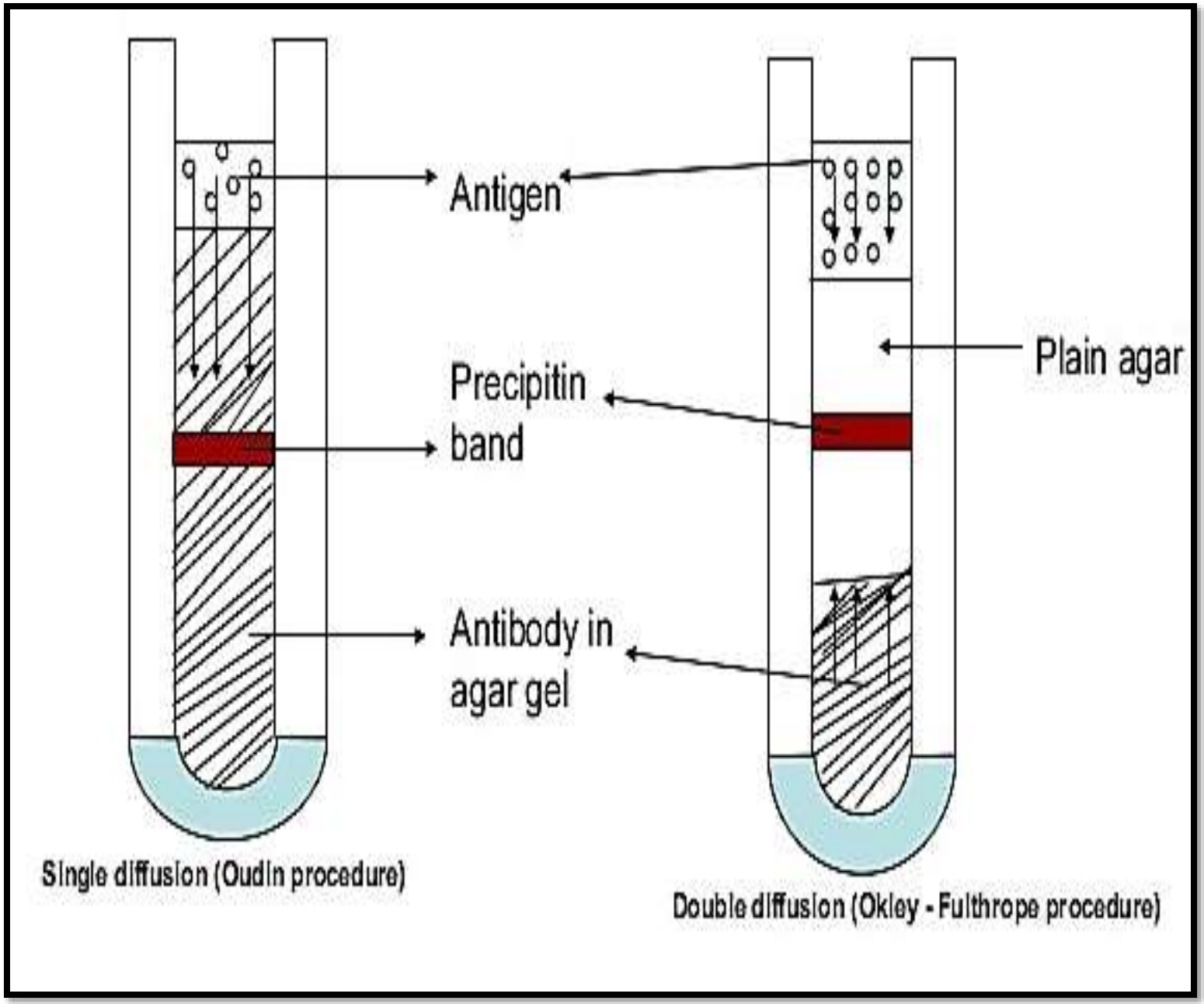


3. DOUBLE DIFFUSION IN ONE DIMENSION

This method is also called 'Oakley-Fulthrope' procedure & is performed in a test tube.

Procedure:

1. Ab's are incorporated in gel in test tube
2. Above which a layer of plain agar is placed.
3. Ag layer is poured on top of this plain
4. Ag's & Ab's moves towards each other through intervening layer of plain agar.
5. Ag & Ab reacts to form precipitin ring at optimum concentration.


Antigen
Precipitin band
Antibody in agar gel
Plain agar
Single diffusion (Oudin procedure)
Double diffusion (Okley - Fulthrope procedure)

4. DOUBLE DIFFUSION IN TWO DIMENSIONS

This method is also called 'Ouchterlony's procedure'. In this both Ag & Ab diffuse independently through agar gel in 2 dimensions i.e. horizontally & vertically (radially)

Procedure:

1. Wells are cut in agar gel poured in glass slide or petri dish
2. Anti-serum consisting of Ab's are poured in the central well
3. Different Ag's are added to it surrounding the central well
4. After incubation the lines of precipitin are formed at the sites of combination of Ag & Ab.

3 types of lines can be formed:

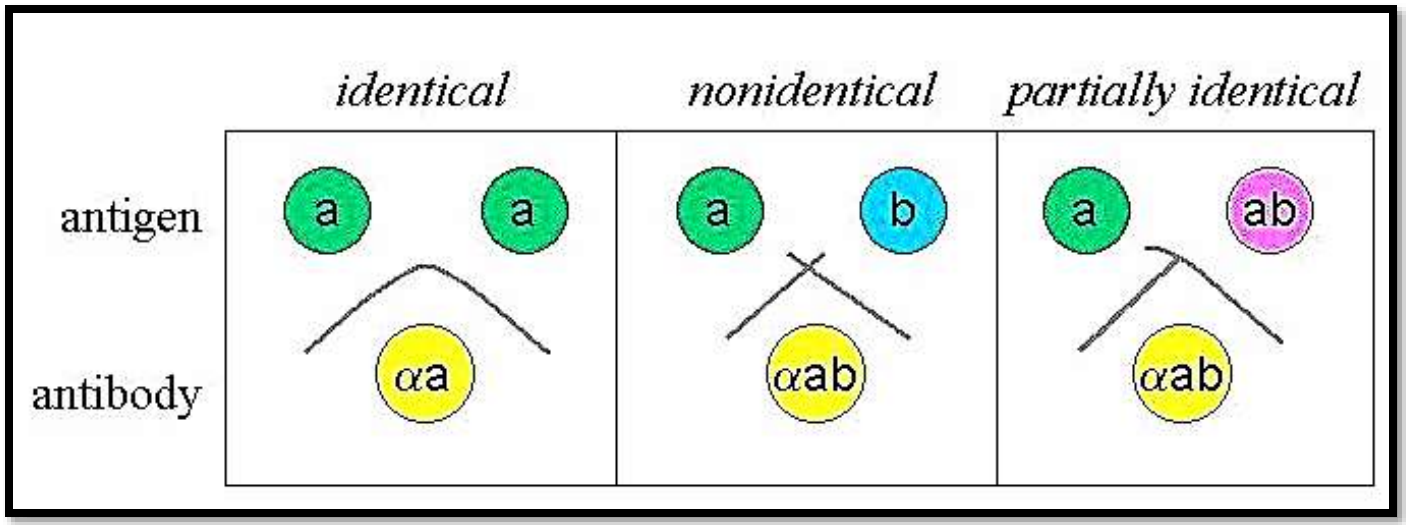
a) At junction forming an arc => presence of common epitope in Ag.

b) Crossed lines => no common epitope b/w compared Ag's

c) Fusion of lines with a spur => cross-reaction or partial identity

USES:

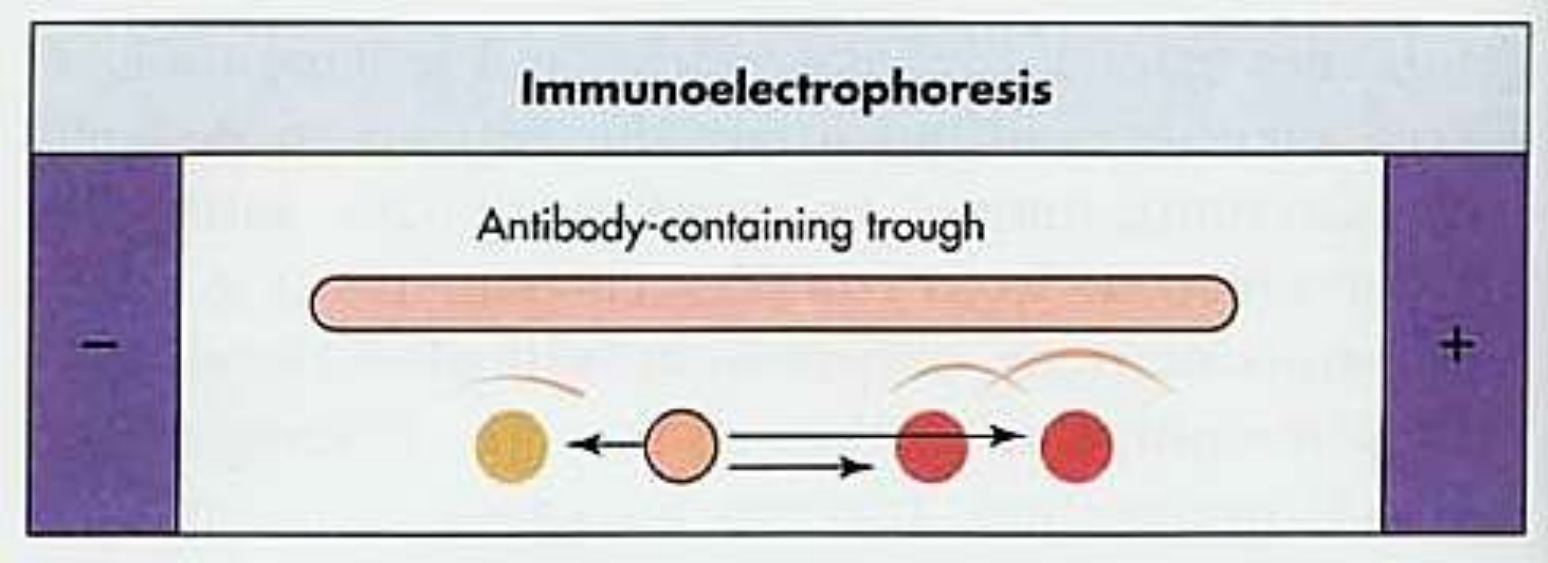
a) Demonstration of Ab's in diagnosis of small pox
b) Identification of fungal Ag's
c) Detection of Ab's to extract nuclear antigens.



IMMUNO-ELECTROPHORESIS

It is a method in which different antigens are separated according to their charge by the presence of electrical field.

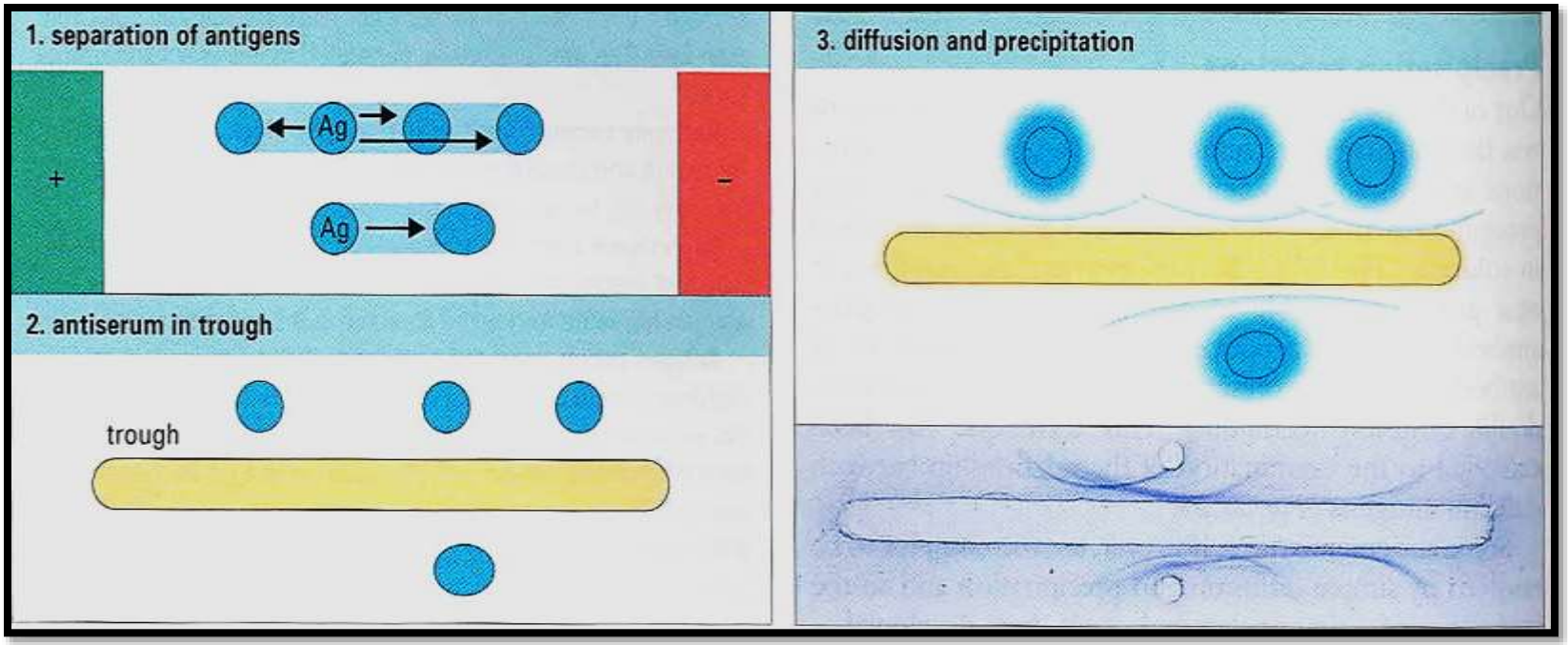
It is a process of combination of immunodiffusion & electrophoresis.



Procedure:

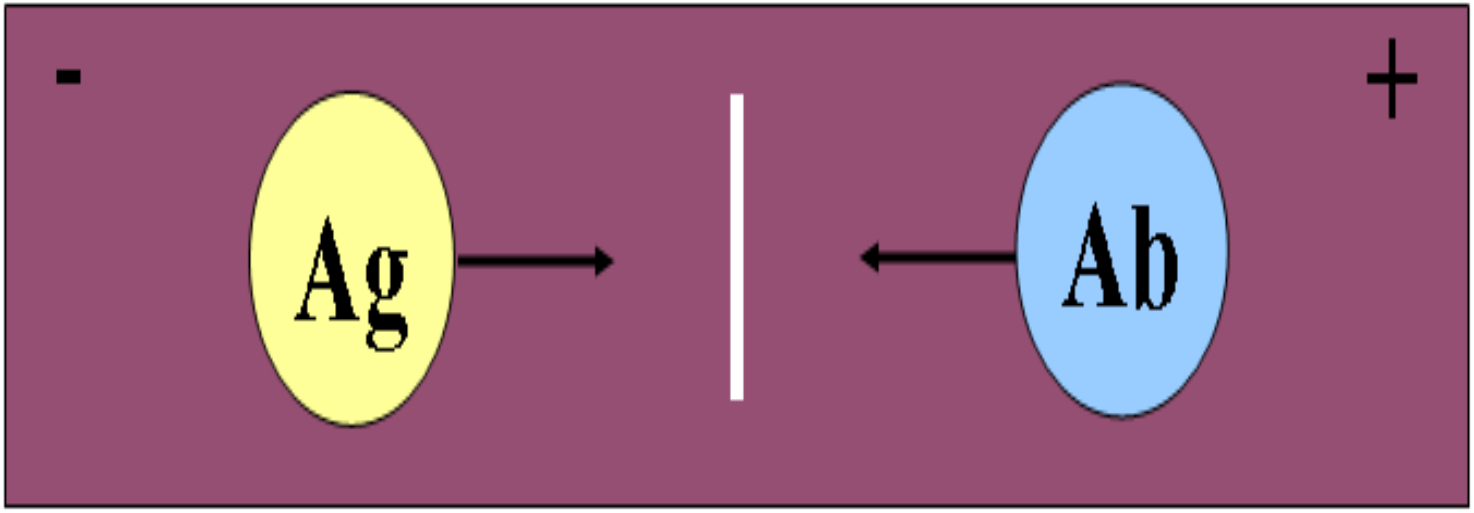
1. A drop of Ag is placed into a well in agar on glass slide.
2. Electric current is passed through agar
3. Ag move in the electric field according to their size & charge.
4. A trough is cut into agar & Ab is poured to it & diffusion is allowed to occur.
5. As the Ag & Ab diffuse they form series of lines

ADVANTAGE -> Number of Ag's can be identified in serum.



COUNTER-CURRENT IMMUNOELECROPHORESIS

It depends upon the movement of antigen towards the anode (positive) & antibody towards the cathode (negative) through agar in the electric field.



Procedure:

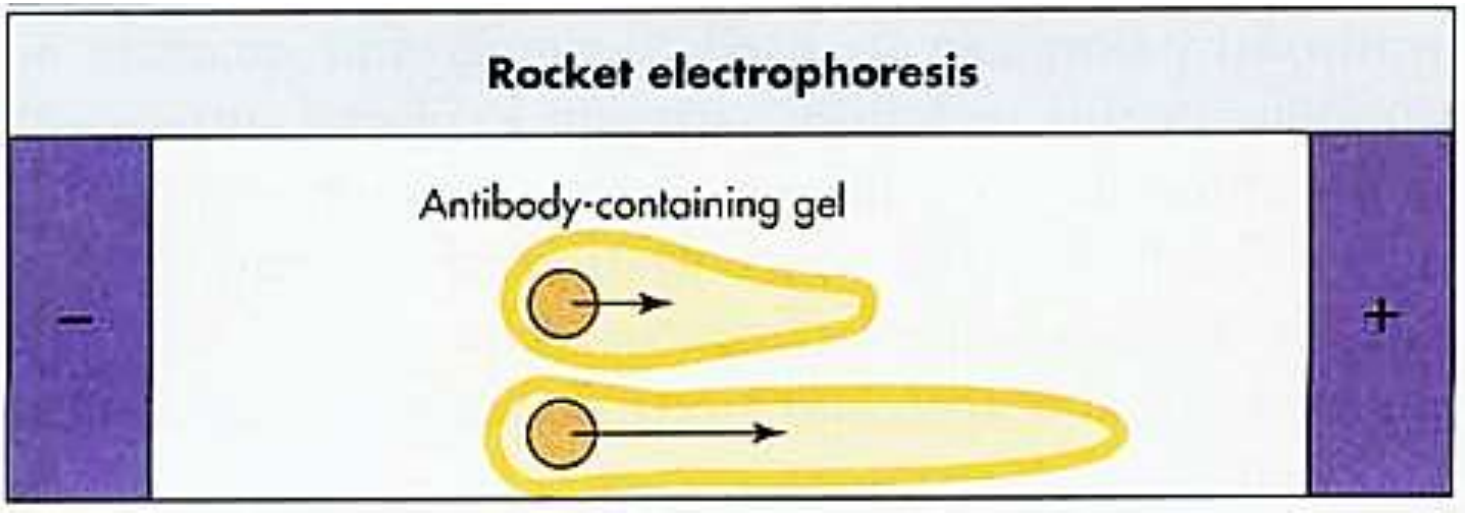
1. Performed on glass slide with agarose in which pair of wells are punched.
2. One is filled with Ag & the other with the Ab
3. Electric current is passed
4. Migration is visible in 30-60 mins.
5. It is a rapid & highly specific method for the detection of Ag & Ab in serum, CSF, other body fluids for detection of diseases.

USE -> Commonly used for hepatitis-B surface antigen.

ROCKET ELECTROPHORESIS

It is an adaptation of radial immunodiffusion developed by 'Laurel'.

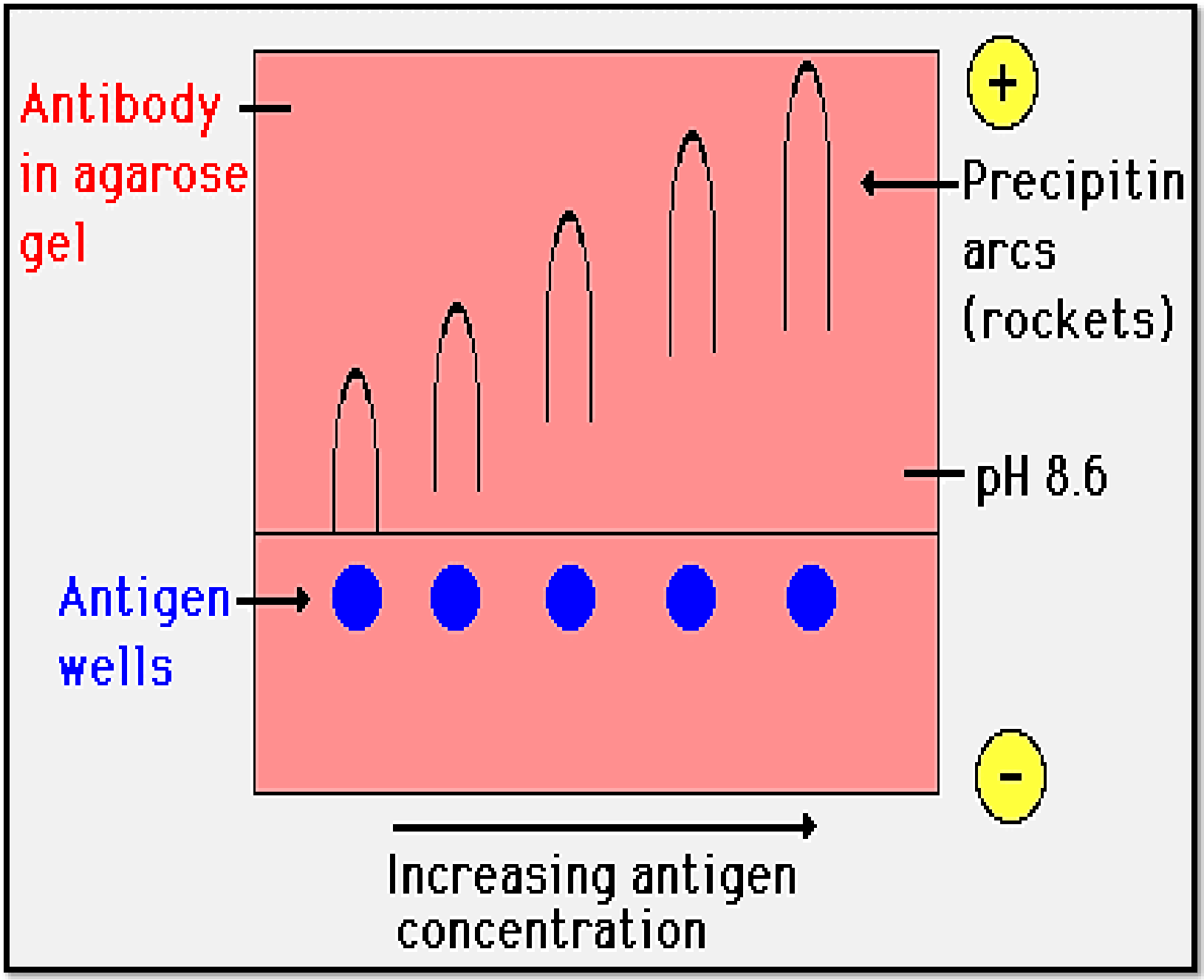
It is called so due to the appearance of precipitin bands in the shape of cone-like structures (rocket appearance) as the end result.



Procedure:

1. Ab is incorporated in the gel, Ag's are placed in wells cut in gel
2. Electric current is passed, which facilitated migration of Ag into agar
3. This results in formation of precipitin conical in shape, resembling rocket.
4. The height of rocket is directly proportional to concentration of antigen.

USE -> For quantitative estimation of antigen in serum


Antibody in agarose gel
Precipitin arcs (rockets)
pH 8.6
Antigen wells
+
-
Increasing antigen concentration

CONCLUSION

Thus we hereby conclude with the fact that antigen-antibody reactions are very important for serological testing of human beings, as they give you a complete picture of all the immune responses occurring the body & helps determining the immunological disorders by the antigen (either self or non-self).


THANK
YOU!