BHARATHIDASAN UNIVERSITY

Tiruchirappalli – 620 024,
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Programme: M.Sc., Biotechnology (Marine)

Course Title : Immunology

Course Code : 21 CC7

Unit: III
Antigen and Antibody Interaction

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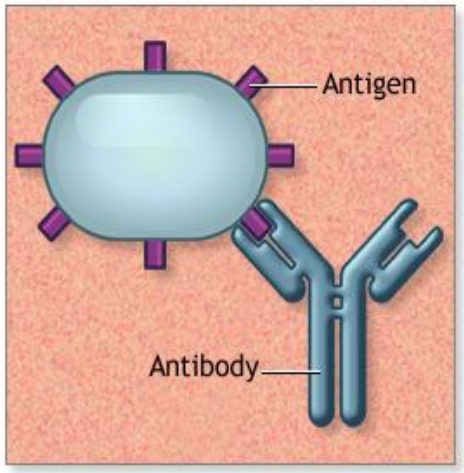
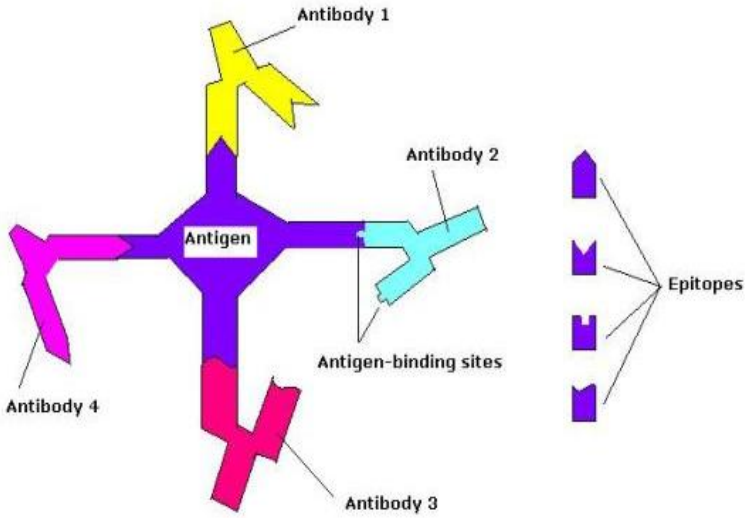
Antigen(Ag) – Antibody(Ab) & the Forces of Attraction



An antigen is a substance that induces the formation of antibodies because it is recognized by the immune system as a threat.



Significance of the forces of attraction :

1.Stability: Forces stabilize the antigen-antibody complex for effective pathogen neutralization.

2. Specificity: Forces ensure selective binding of antibodies to specific antigens.

3. Recognition and Affinity: Forces contribute to high affinity, crucial for antigen recognition and neutralization.

4. Functional Immune Response: Forces enable subsequent immune functions like opsonization and complement activation.

5.Reversibility and Regulation: Non-covalent forces allow reversible interactions, crucial for dynamic immune regulation.

Forces acting behind Ag-Ab interaction

The interaction between an antibody and an antigen depends on **FOUR** types of NONCOVALENT FORCES :

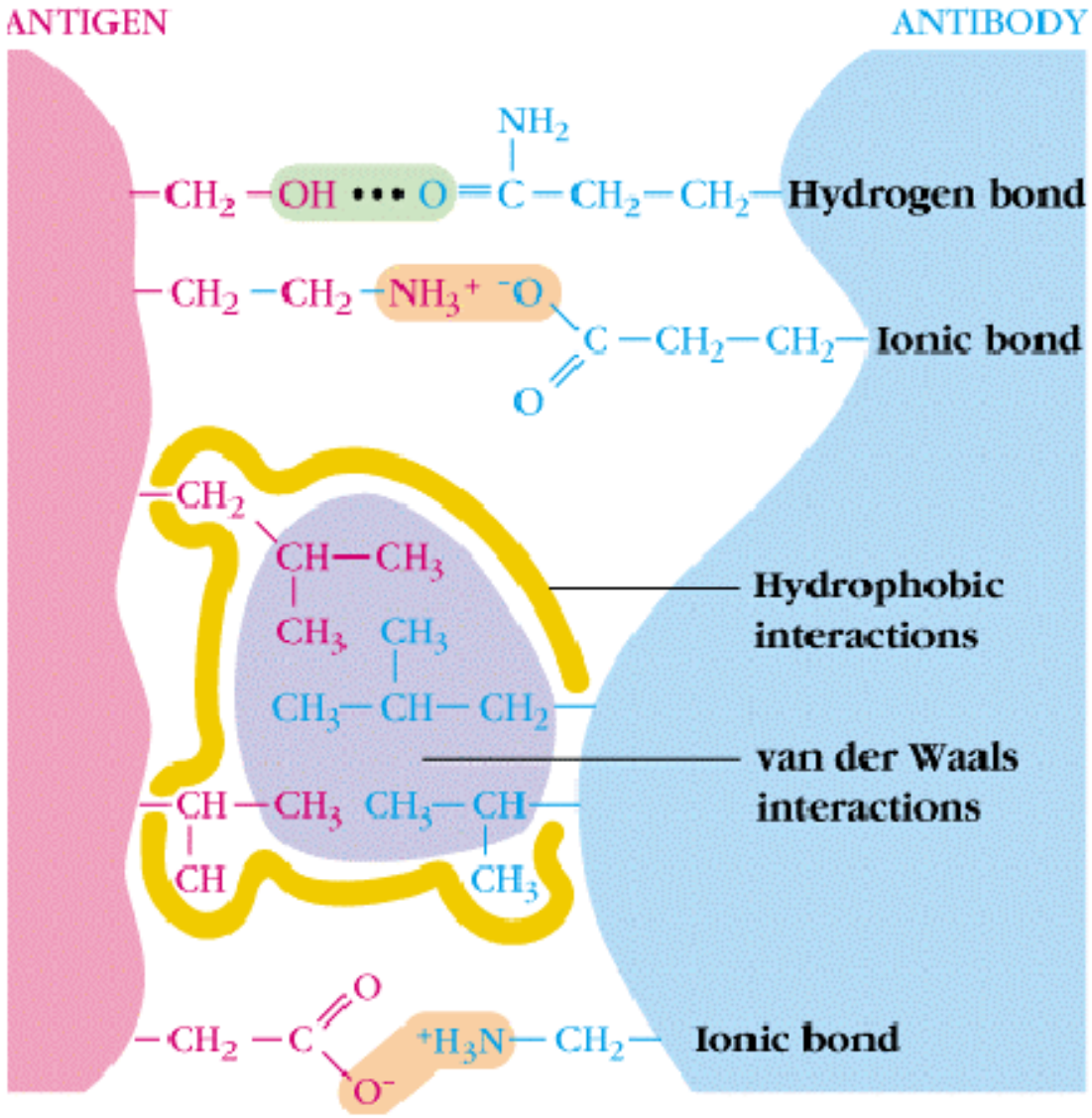
(1) Hydrogen bonds: a hydrogen atom is shared between two electronegative atoms

(2) Ionic bonds: between oppositely charged residues

(3) Hydrophobic interactions: in which water forces hydrophobic groups together

(4) Van der Waals interactions: between the outer electron clouds of two or more atoms.

In an *aqueous environment, noncovalent interactions are extremely weak* and depend upon close complementarity of the shapes of antibody and antigen.



Factors affecting Ag-Ab Interactions

TEMPERATURE : H-bonds are stable at *low* temperatures and Hydrophobic bonds are stable at *high* temperatures

pH : Optimal pH range is 6.5 to 8.5

NO. OF AG-AB BINDING SITES : IgM (pentamer) will bind more efficiently with antigens than IgG (monomer)

STRUCTURAL ARRANGEMENT : Favours interaction between epitope and paratope if it fit as lock and key

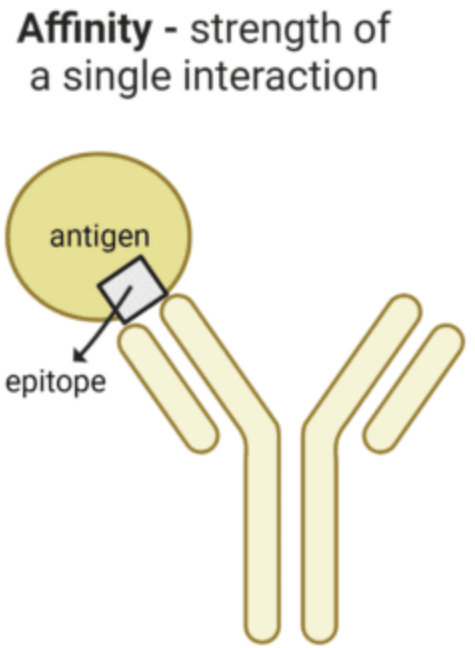
IONIC STRENGTH : Important in blood group serology. Here the reaction is significantly influenced by sodium and chloride ions.

ENZYME TREATMENT : papain, ficin, bromelin

CONCENTRATIONS OF Ag, Ab : Increase in the concentration of antigen and antibody enhances the reaction

AFFINITY

The combined strength of the noncovalent interactions between a single antigen-binding site on an antibody and a single epitope is the **affinity** of the antibody for that epitope.



- *Low-affinity antibodies* bind antigen *weakly* and tend to dissociate readily
- *High-affinity antibodies* bind antigen *more tightly* and remain bound longer
- Operates over *very short distance* (1×10^{-7}). Requires high degree of complementarity between antigen and antibody.

Antibody Affinity : A Quantitative Measure of Binding Strength

Association between : 1 binding site of Ab-Monovalent Ag : *Equation*

$$Ag + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ag\text{-}Ab$$

k1 = forward (association) rate constant
k-1 = reverse (dissociation) rate constant

*K*a = *k*1/*k*-1 , *K*a = Equilibrium constant

K= *K*a / *K*d
K = binding affinity

$$K = \frac{[Ag\text{-}Ab]}{[Ab][Ag]}$$

<u>Calculation of *K*a :</u>

Molar conc. of bound Ag-Ab complex : Molar conc. of unbound Ag & Ab at equilibrium

$$K_a = \frac{[Ag\text{-}Ab]}{[Ab][Ag]}$$

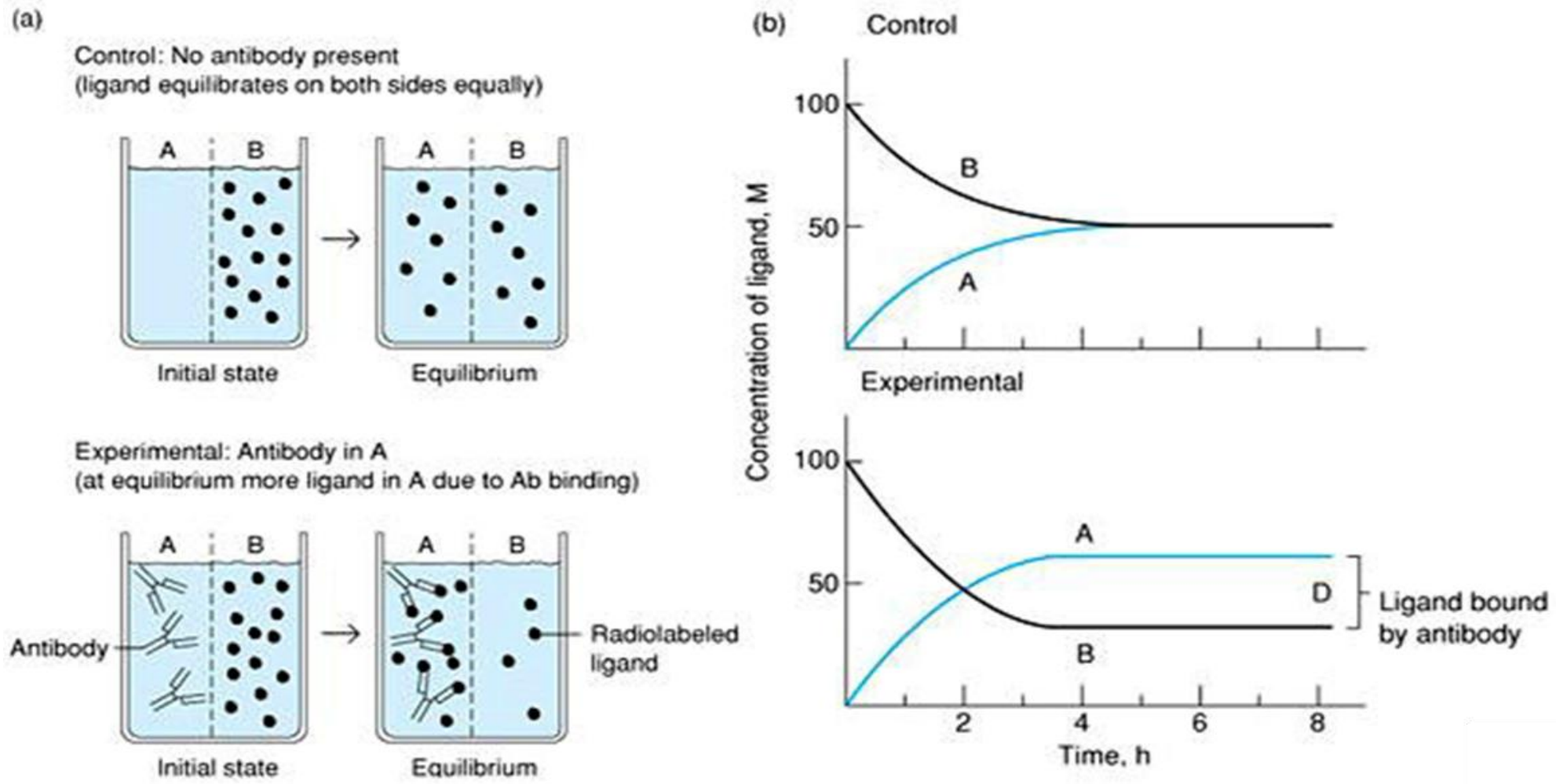
Dissociation of Ag-Ab Complex :

$$Ag\text{-}Ab \rightleftharpoons Ab + Ag$$

*K*d = Dissociation constant

$$K_d = [Ab][Ag]/[Ab\text{-}Ag] = 1/K_a$$

Equilibrium Dialysis : The Way to Determine *K*a Value



Scatchard equation : *helping to determine the affinity of a receptor for a ligand and the number of binding sites*

Total concentration of Ab in the equilibrium dialysis chamber is known, the equilibrium equation can be written as :

$K_a = [Ab\text{-}Ag]/[Ab][Ag] = r/c(n-r)$

Where,

r = ratio of the concentration of bound ligand to total antibody concentration

c = concentration of free ligand

n = number of binding sites per antibody molecule

This can be rearranged to give the ***Scatchard Equation*** :

$r/c = K_a n - K_a r$

AVIDITY

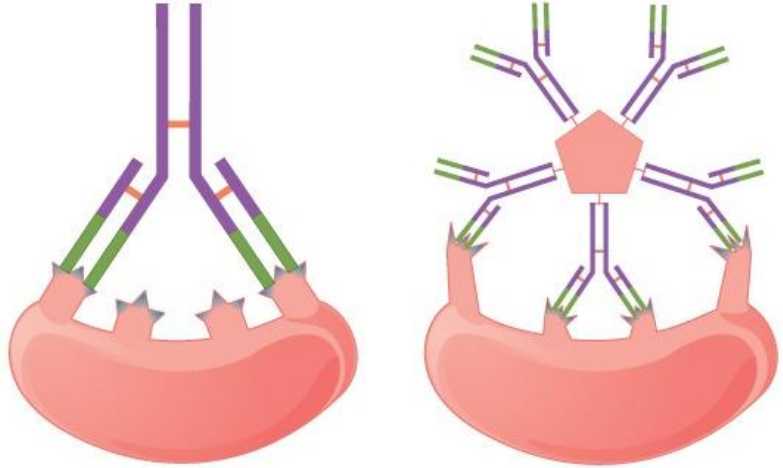
Avidity refers to the accumulated strength of *multiple* affinities of individual non-covalent binding interactions, such as between a protein receptor and its ligand, and is commonly referred to as functional affinity.

- The avidity of an antibody is a *better measure of its binding capacity* within biological systems than the affinity of its individual binding sites.
- High avidity *can compensate for low affinity*.

For example, IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.

Factors affecting Avidity:

1. Affinity of Individual binding sites
2. Valency
3. Ag structure
4. Ab structure
5. Concentration of Ag & Ab
6. Environmental Conditions
7. Glycosylation
8. Steric Factors
9. Cross linking & Aggregation

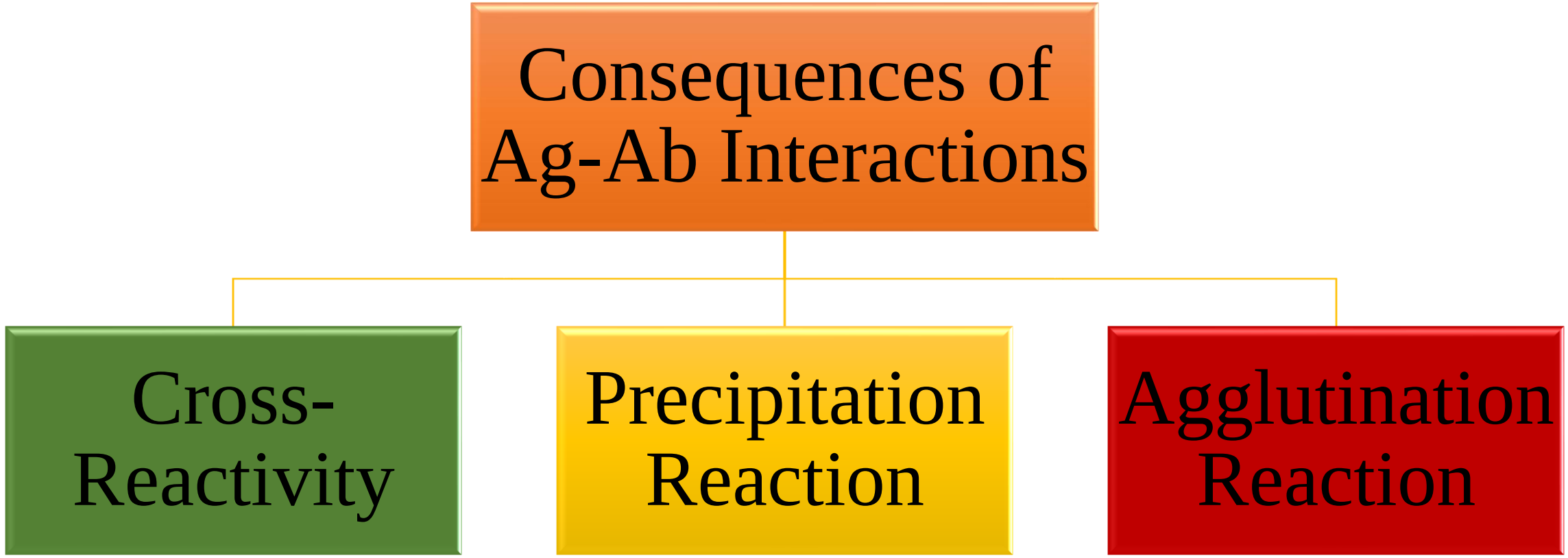


Affinity refers to the strength of a single antibody–antigen interaction. Each IgG antigen binding site typically has high affinity for its target.

Avidity refers to the strength of all interactions combined. IgM typically has low affinity antigen binding sites, but there are ten of them, so avidity is high.

Affinity Vs Avidity

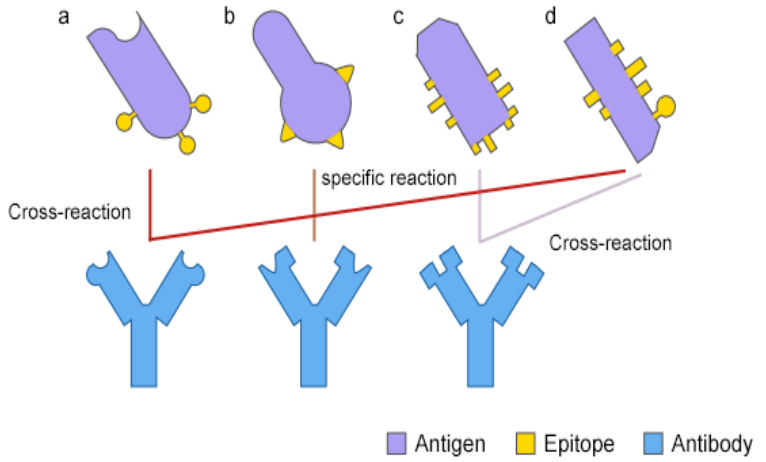
Factor	Affinity	Avidity
Definition	Strength of binding between a single antigen-binding site on an antibody and its specific epitope on an antigen.	Overall strength of binding between an antibody with multiple binding sites and a multivalent antigen.
Binding Site	Single binding site on the antibody.	Multiple binding sites on the antibody.
Measurement	Measured for individual antigen-antibody interactions.	Measured for cumulative interactions of multiple binding sites.
Representation	Quantified as an equilibrium constant (Ka or Kd).	Represents the functional binding strength in a biological context.
Influence	Affected by intrinsic factors like hydrogen bonds, van der Waals forces, and ionic bonds at a single site.	Affected by the sum of multiple affinities, spatial arrangement of epitopes, and antibody structure.
Antibody Types	IgG typically has higher affinity at each site.	IgM typically has higher avidity due to its pentameric structure.
Stability	Describes the stability of a single antibody-antigen complex.	Describes the overall stability of the antibody-antigen interaction involving multiple complexes.
Biological Significance	Important for understanding specific interactions between antibodies and antigens.	Crucial for understanding the effectiveness of antibody binding in complex biological systems.
Examples	Binding of a monoclonal antibody to a single epitope.	Binding of an IgM antibody to a pathogen with multiple identical epitopes.


Consequences of Ag-Ab Interactions
Cross-Reactivity
Precipitation Reaction
Agglutination Reaction

CROSS REACTIVITY

Cross-reactivity in immunology refers to the ability of an antibody to react with similar but distinct antigens, not just the specific antigen that induced its production.

Cross-reactivity is often observed among polysaccharide antigens that contain similar oligosaccharide residues. The ABO blood-group antigens, for example, are glycoproteins expressed on red blood cells.



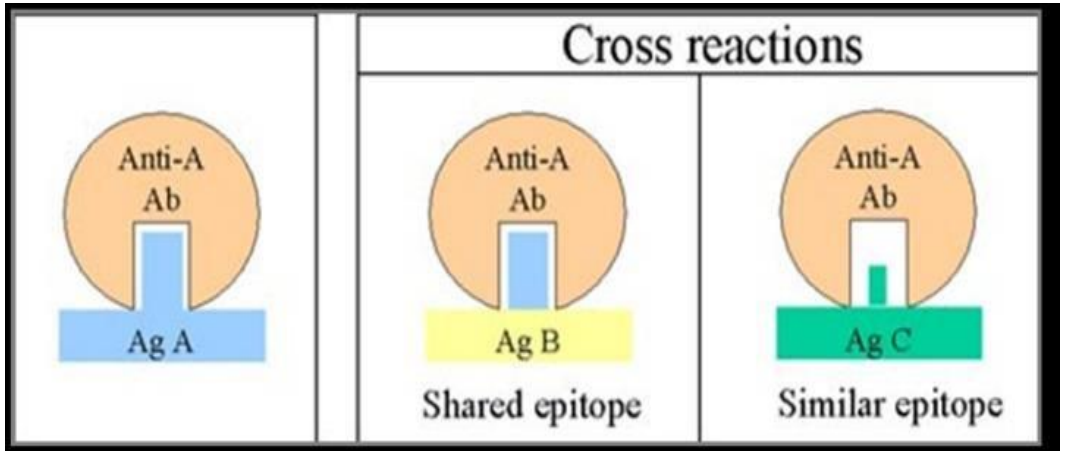
Scenario	Antigens	Antibodies Produced	Cross-Reactivity
Type B Blood Host	B antigens on RBCs	Anti-A antibodies	Anti-A antibodies react with A antigens (from bacteria and foreign RBCs)
Bacterial Antigens	Similar to A antigens	Anti-A antibodies	Anti-A antibodies bind to A-like antigens on bacteria

Blood Type and Antibodies:

- **Type A**: A antigen on RBCs, anti-B antibodies in plasma.
- **Type B**: B antigen on RBCs, anti-A antibodies in plasma.
- **Type AB**: A and B antigens on RBCs, no anti-A or anti-B antibodies in plasma.
- **Type O**: No A or B antigens on RBCs, anti-A and anti-B antibodies in plasma.

- Cross-reactivity is often observed among polysaccharide antigens that contain similar oligosaccharide residues.
- The ABO blood-group antigens, for example, are glycoproteins expressed on red blood cells.
- Subtle differences in the terminal residues of the sugars attached to these surface proteins distinguish the A and B blood-group antigens.
- An individual lacking one or both of these antigens will have serum anti bodies to the missing antigen(s).
- The antibodies are induced not by exposure to red blood cell antigens but by exposure to cross-reacting microbial antigens present on common intestinal bacteria. These microbial antigens induce the formation of antibodies in individuals lacking the similar blood-group antigens on their red blood cells.

- Can induce tissue damaging autoimmune reaction
- Example : Antigen M of *Streptococcus pyogenes*
- Vaccinia virus expresses cross-reacting epitopes with Variola virus



PRECIPITATION REACTION

Ab and soluble Ag interacting in aqueous solution form a lattice that eventually develops into a visible precipitate.

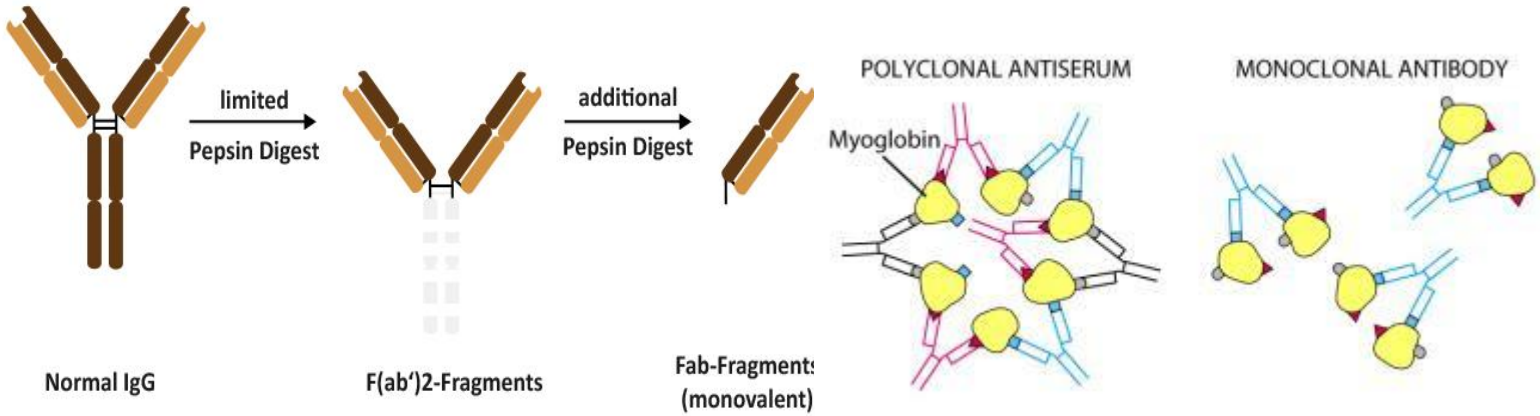
Antibodies that aggregate soluble antigens are called precipitins.

Ag-Ab complexes = in minutes

Formation of precipitin lines = slowly (1-2 days)

Formation of an Ag-Ab lattice depends on the Valency of both Ab and Ag :

1. The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments
2. The antigens must be either bivalent or polyvalent



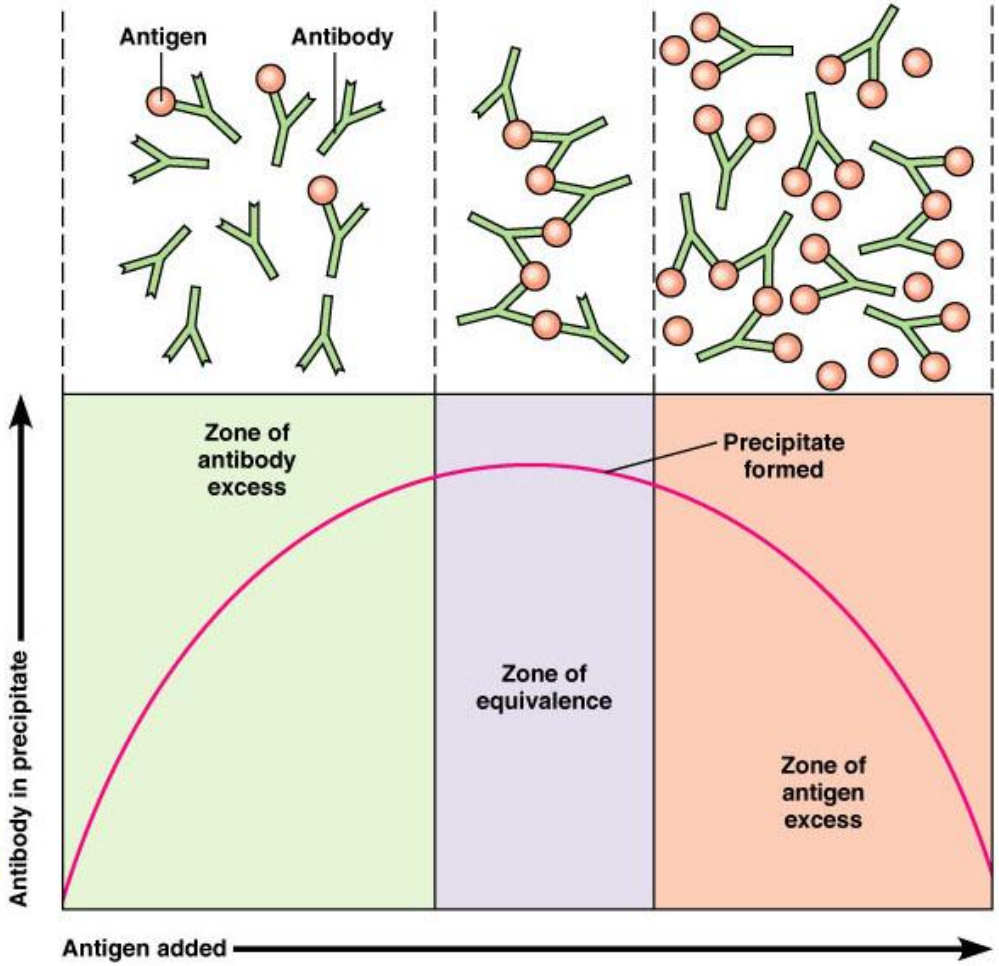
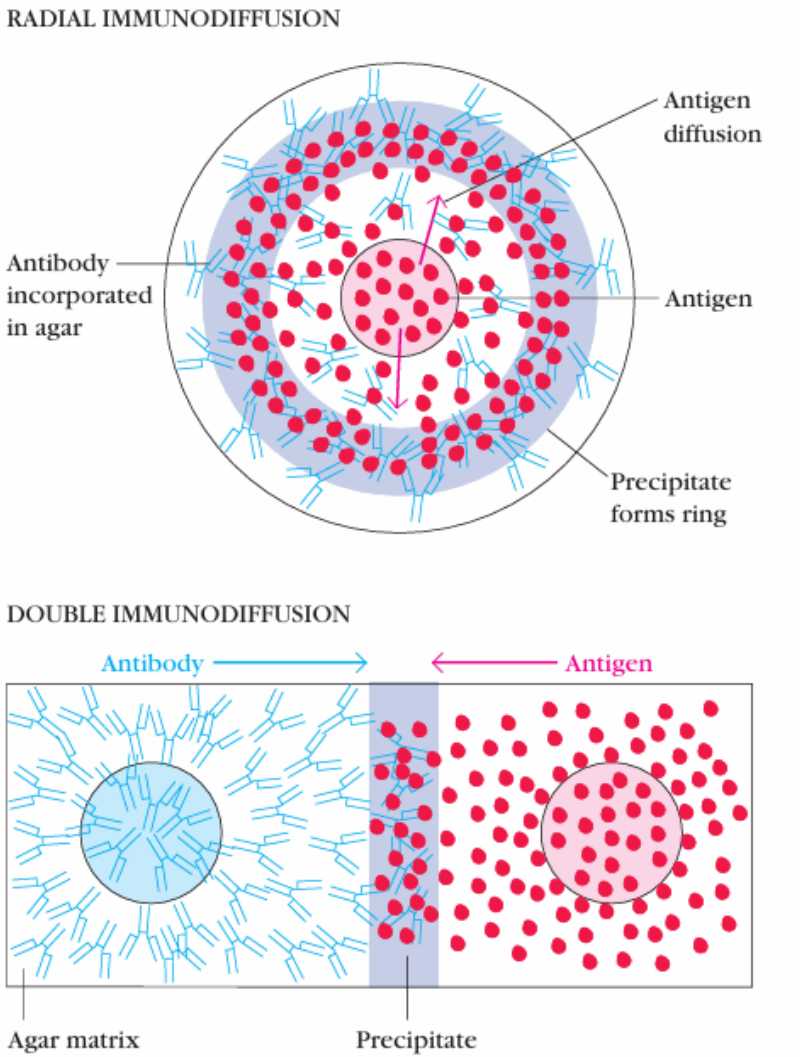
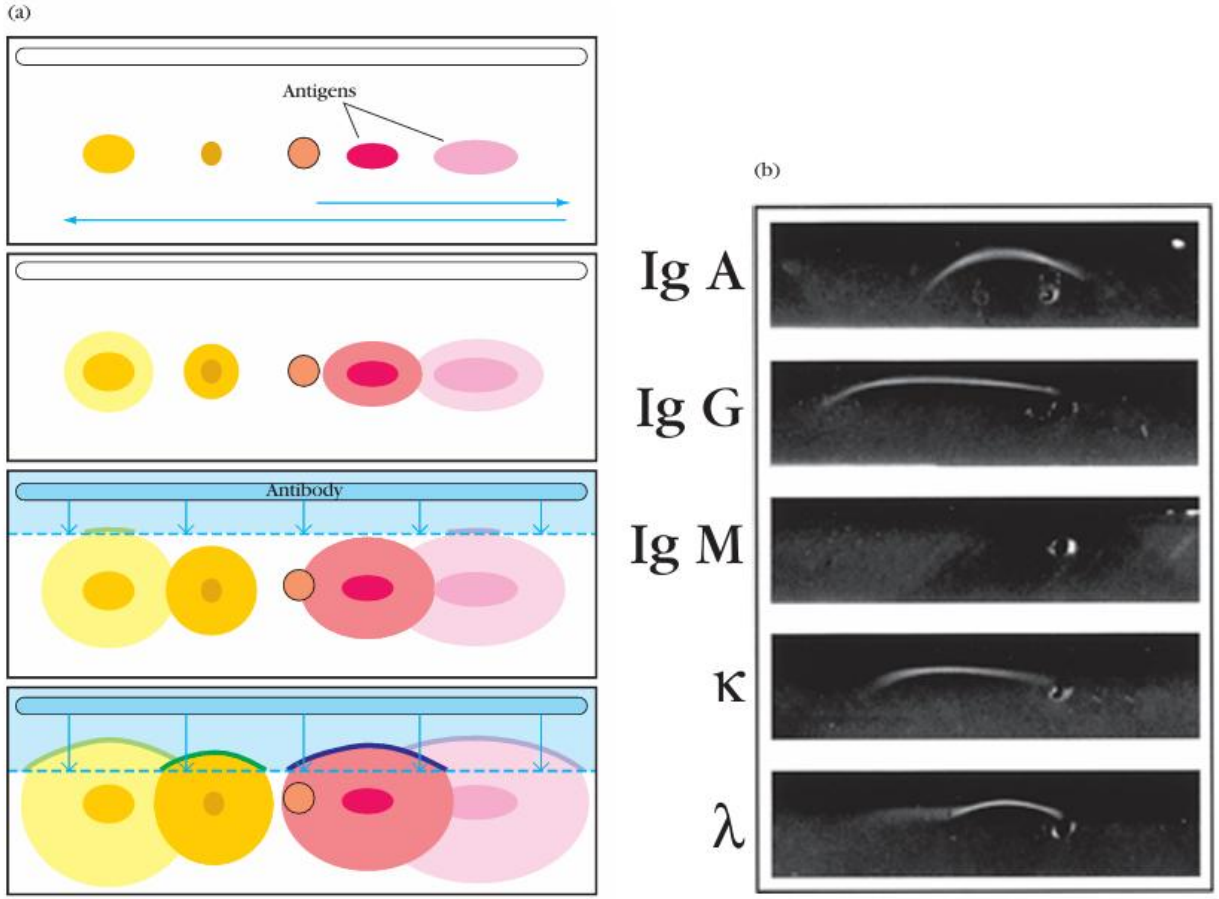


Fig: Precipitation Reaction in Solution

TYPES OF PRECIPITATION REACTIONS

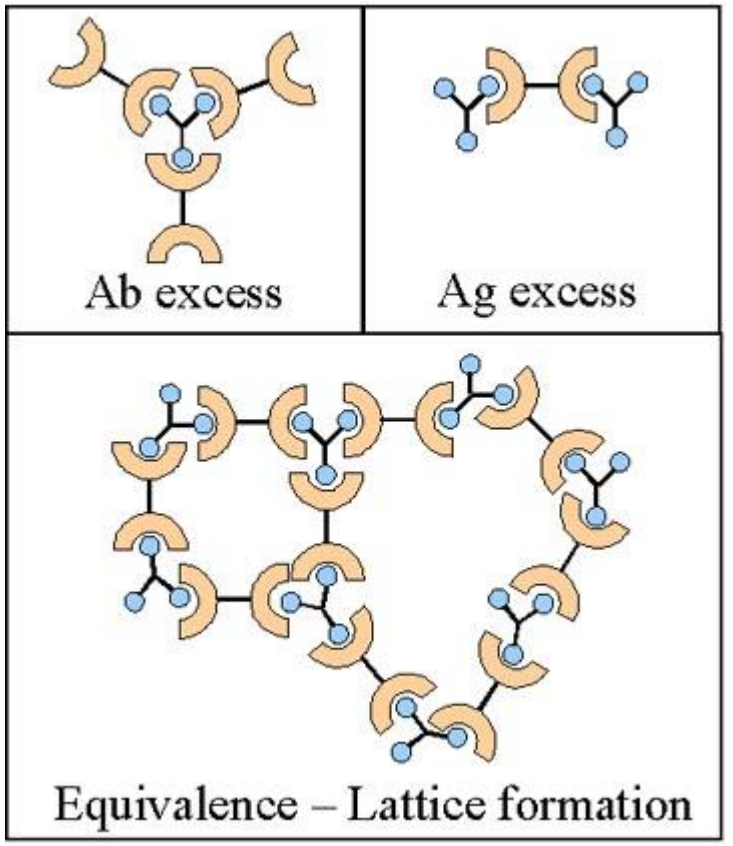




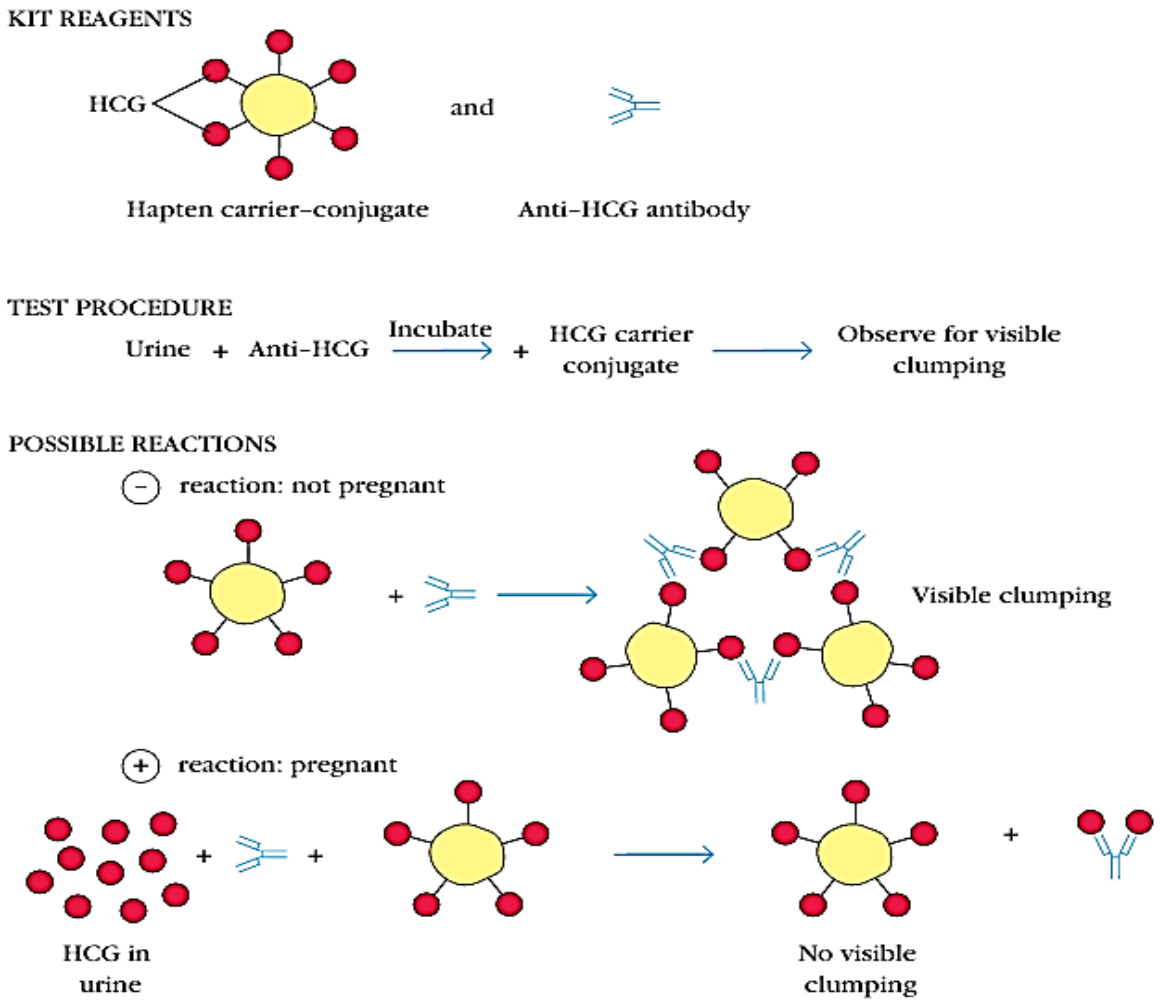
Immunoelectrophoresis

AGGLUTINATION REACTION

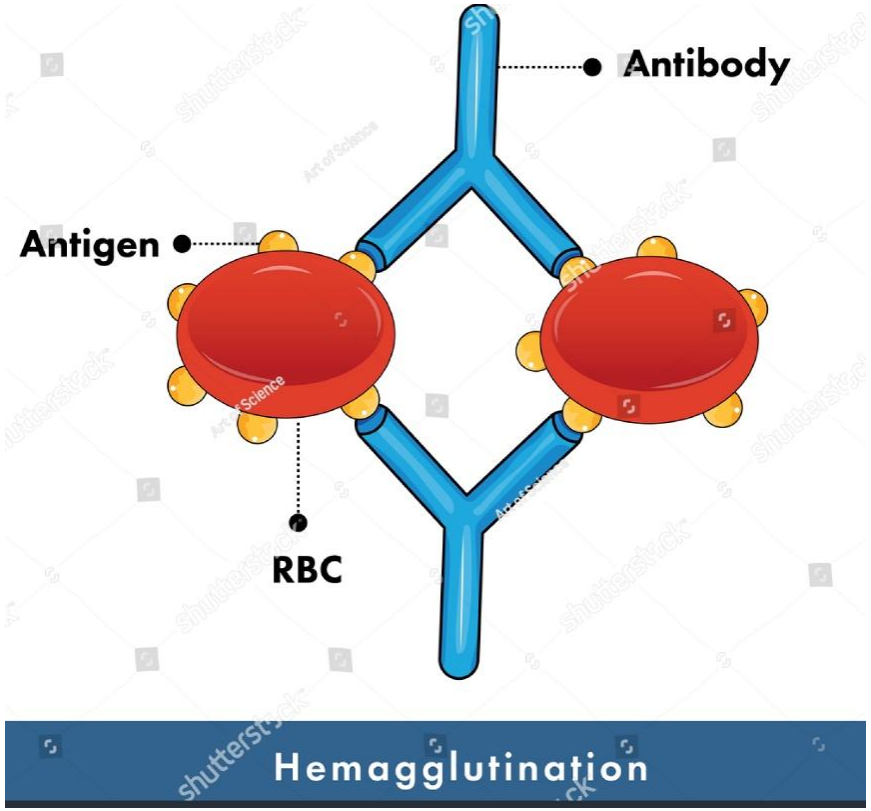
- The interaction between antibody and a particulate antigen results in visible clumping called agglutination.
- Antibodies that produce such reactions are called agglutinins.
- Agglutination reactions are similar in principle to precipitation reactions; they depend on the crosslinking of polyvalent antigens.
- Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the **prozone** effect.



AGGLUTINATION REACTION



a. Agglutination Inhibition- Absence of Agglutination is Diagnostic of Antigen : Pregnancy Testing Kits



b. Hemagglutination Is Used in Blood Typing