

Immunology Lab(1)

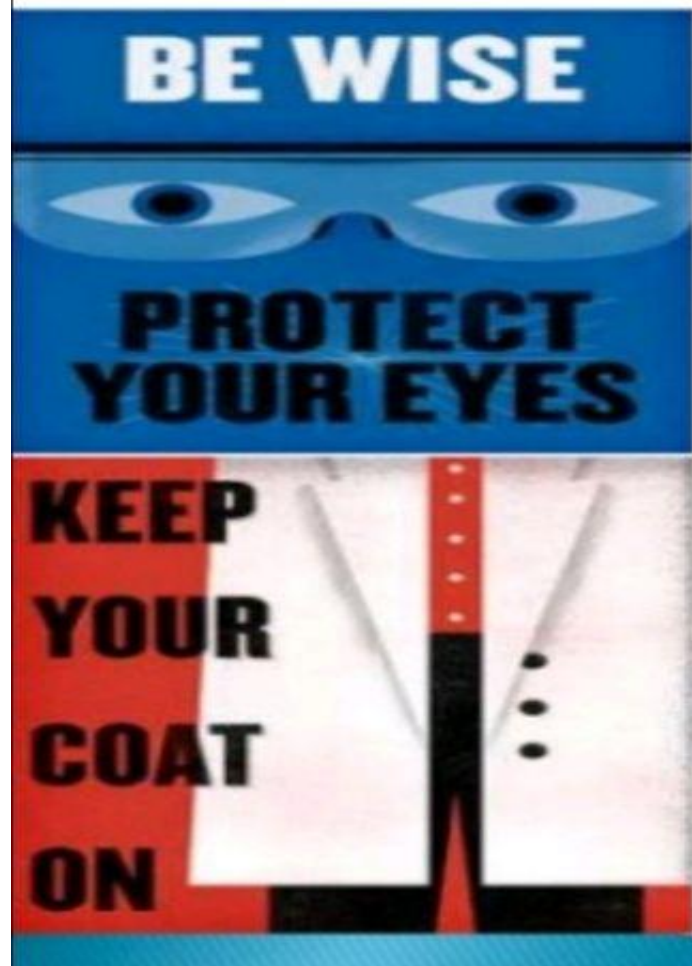
Sample collection, preservation and storage

Presented

by

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SAFETY PROCEDURES IN LABORATORY



Proper Sample Collection:- type of specimen, Suitable Site that taken the specimen from it, Suitable time when the sample taken and Quantity (volume) of specimen.

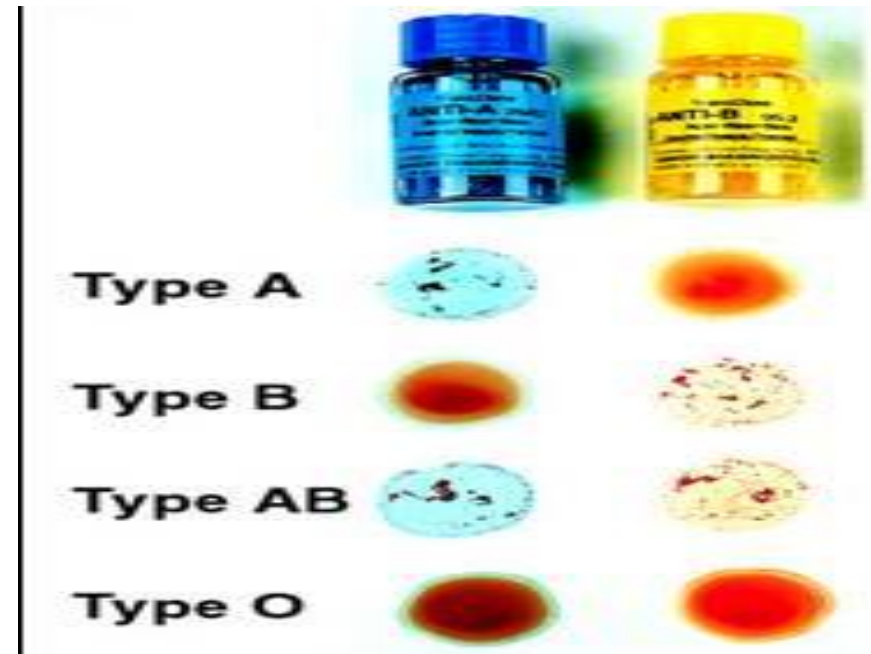
Below the Types of Samples:

- **Blood specimen: Plasma, Serum and PBMCs.**
- **Body Fluids: Urine, Saliva, CSF, Synovial, peritoneal, Pleural, Pericardial and Ascitis Fluid.**
- **Swab: nasopharyngeal Swab, Oropharyngeal swabs**
- **Sputum**
- **Feces**
- **Tissue**

*For immunological (Serological test) , The blood Sample Consider the Major Specimen for indirect Detection of Antibodies in Serum of Patients against Microorganisms such as (Virus, Fungi, Bacteria , Parasite,etc),and Detection of Ags Such as Interleukins.

Collection of Blood Components

Whole blood for Antigens Detection. For selected situations, 1 whole blood may be obtained for detection of ABO Blood groups application.



Blood Sample

2.Serum for Antibodies& Antigens Detection.

A. Collection

Optimal timing

- **Acute.** Acute serum specimens should be collected within one week of symptom onset and submitted as soon as possible.
- **Convalescent.** Convalescent specimens should be collected and submitted at 3-6 weeks after the acute specimen was collected.

Example: Blood specimen for detect (HBV, HCV, HIV, CMV,...) and interleukins.

Blood Sample

Method of Collection: For each serum specimen, collect 5 ml of whole blood into a serum separator tube (SST) from adult. A minimum of 1-2 ml of whole blood is needed for testing of pediatric patients.



Serum Separator Tube (SST): It Contains Two agents ; Silica Particles and a serum Separating gel , the Silica Particles work to activate clotting and cause the blood cells to clump together.

Sample Volume

Adult Draw: 5 ml of whole blood

Pediatric: 2ml

New born: Capillary(0.5CC)

***BLOOD Specimen handling.** Allow whole blood to clot at room temperature for a minimum of 30 minutes and centrifuge after that ,the clear serum collect in anew sterile tube, Label the tube with the patient's name, ID number, specimen type, and date collected. Store refrigerated at 4°C or frozen.



Blood Sample

Phlebotomy: is the practice of drawing blood from patients and taking the blood specimens to Lab to prepare the test.

Avoid used the Hemolyzed Blood Sample, it Cause Complement Activation



IMMUNOLOGICAL DIAGNOSIS

Detection of Antibodies

Detection of Antigens

Principle of Immunological Diagnosis

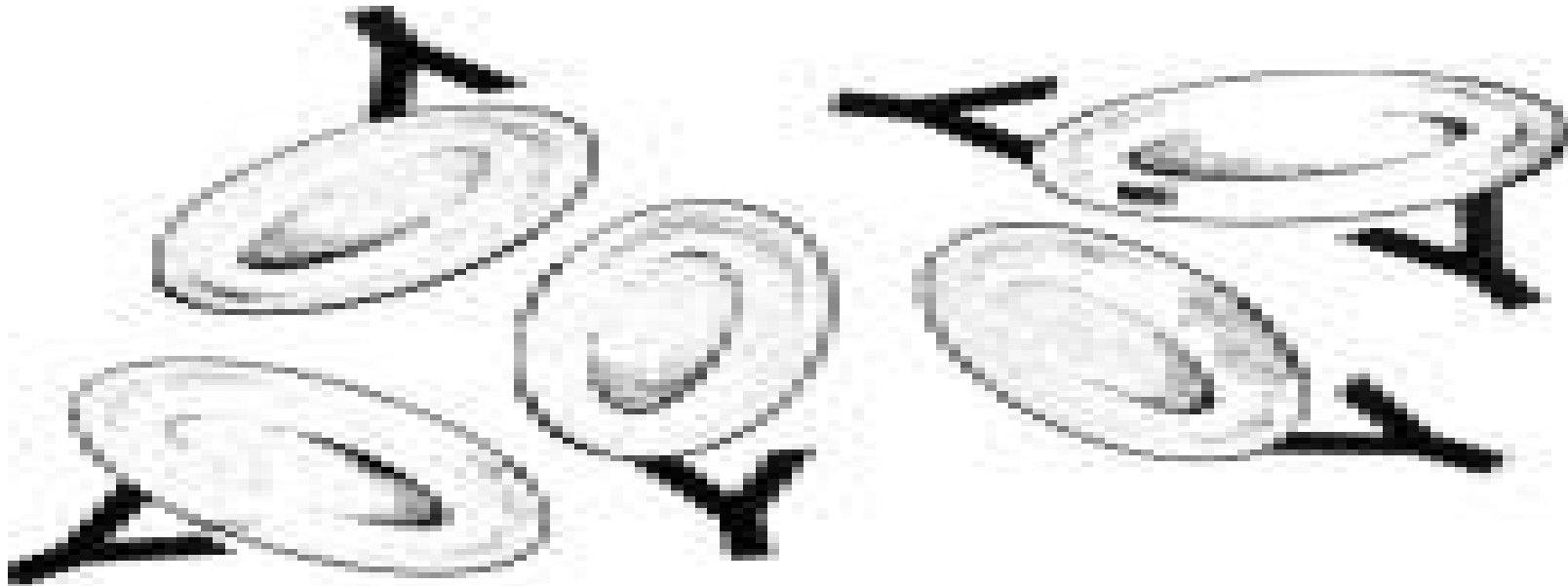
- Detection of antigen/antibody reactions difficult
- Sensitization is the binding of a specific antibody to its' specific antigen
- Cannot be visualized
- Multitude of laboratory methods have been developed to make this visible

Three Distinct Phases of Antigen/Antibody Reactions

- Primary Phenomenon – Sensitization
- Secondary Phenomenon – Lattice formation
- Tertiary Phenomenon – Detected by affect on tissues or cells.

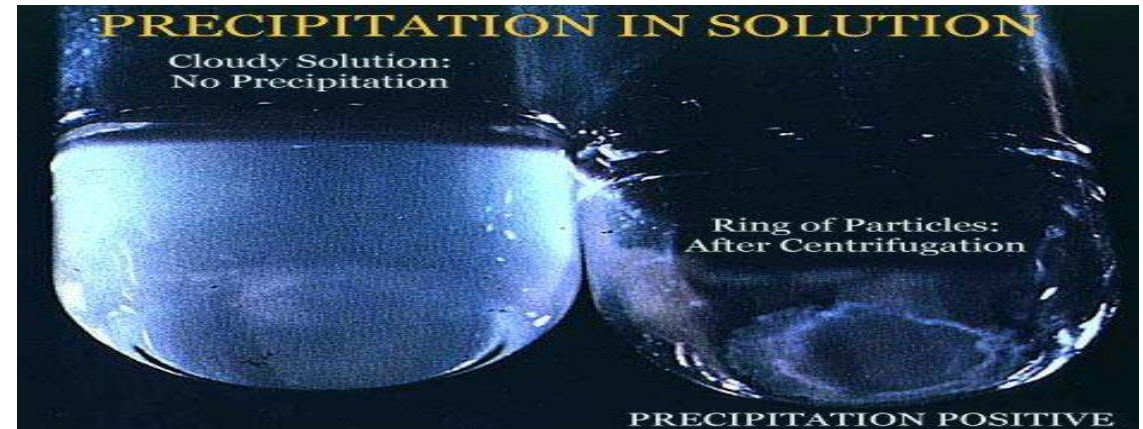
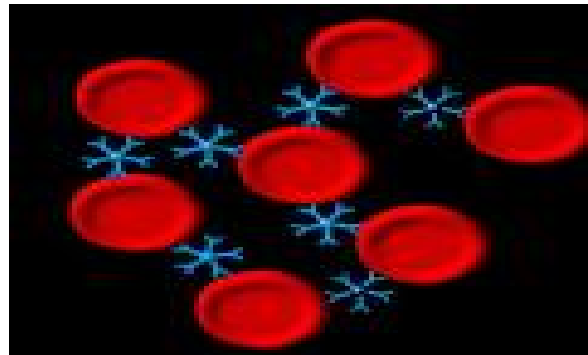
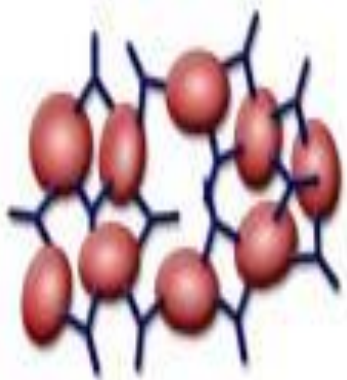
Primary phenomenon

- Sensitization – binding of antibody to antigen – not visible



Secondary Phenomenon

- Lattice Formation
- The Fab portion of the Ig molecule attaches to antigens on 2 adjacent cells-visible results in *agglutination*
- If both antigen and antibody are SOLUBLE reaction will become visible over time, ie, *precipitation*
- <http://www.cehs.siu.edu/fix/medmicro/agabx.htm>



Tertiary Phenomenon

- Reaction not visible, detected by affect of reaction on tissues or cells.
- <http://www.cellsalive.com/mac.htm>

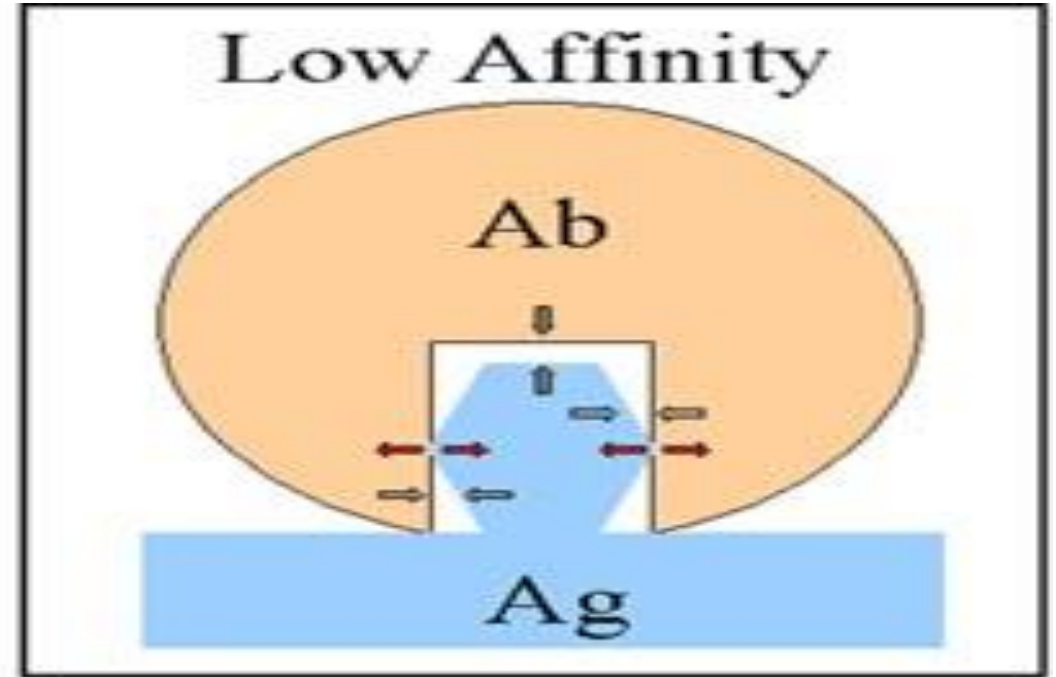
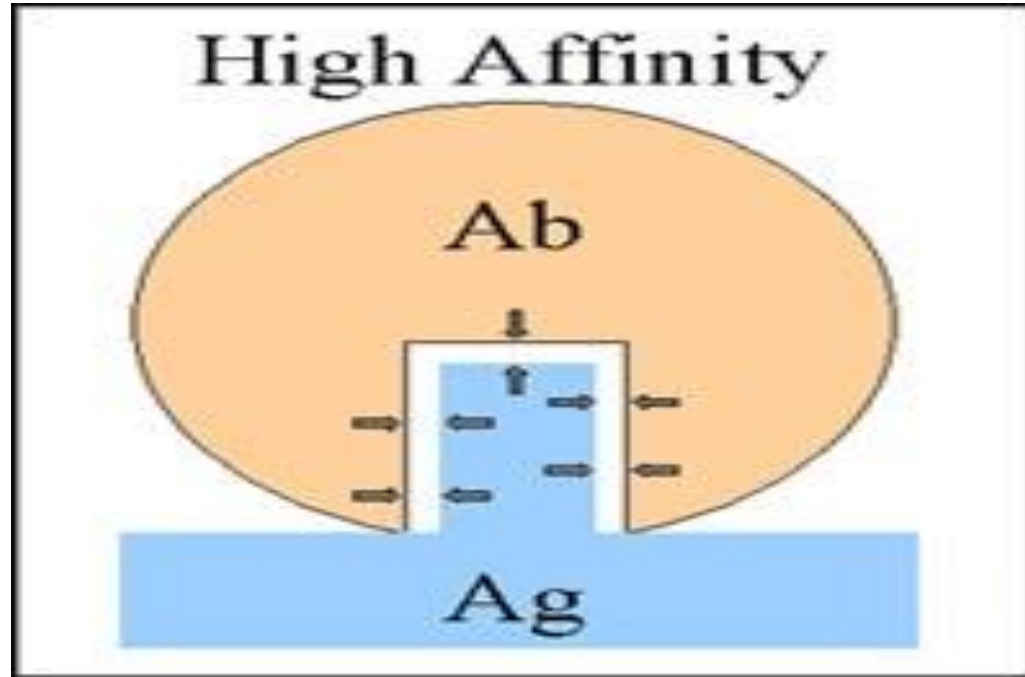
Antigen-Antibody Binding

- Affinity
- Avidity
- Law of Mass Action

Affinity

- Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody.
- It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site .
- Affinity is the equilibrium constant that describes the Ag-Ab reaction as illustrated in Figure 3. Most antibodies have a high affinity for their antigens.


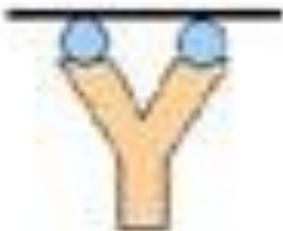
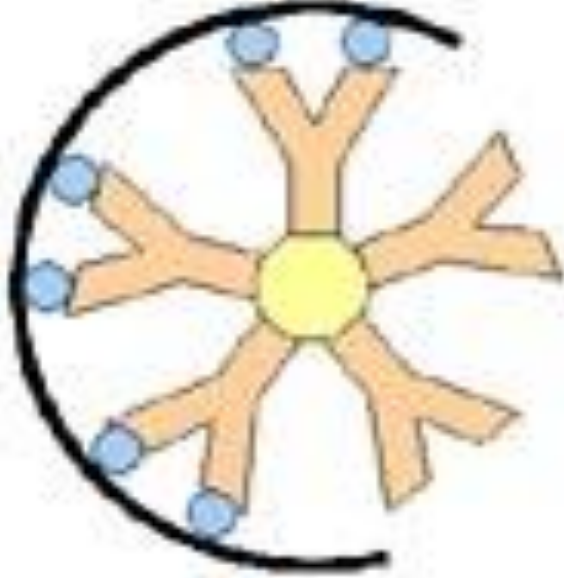
Affinity



Avidity

- Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies.
- Affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.
- Avidity is influenced by both the valence of the antibody and the valence of the antigen.
- Avidity is more than the sum of the individual affinities.

Avidity

 <p>$K_{eq} = 10^4$ Affinity</p>	 <p>10^6 Avidity</p>	 <p>10^{10} Avidity</p>
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Law of Mass Action

- Governs the reversibility of the antigen-antibody reaction.
- Reversible reaction, visible reaction occurs when the rate of binding exceeds the rate of dissociation.



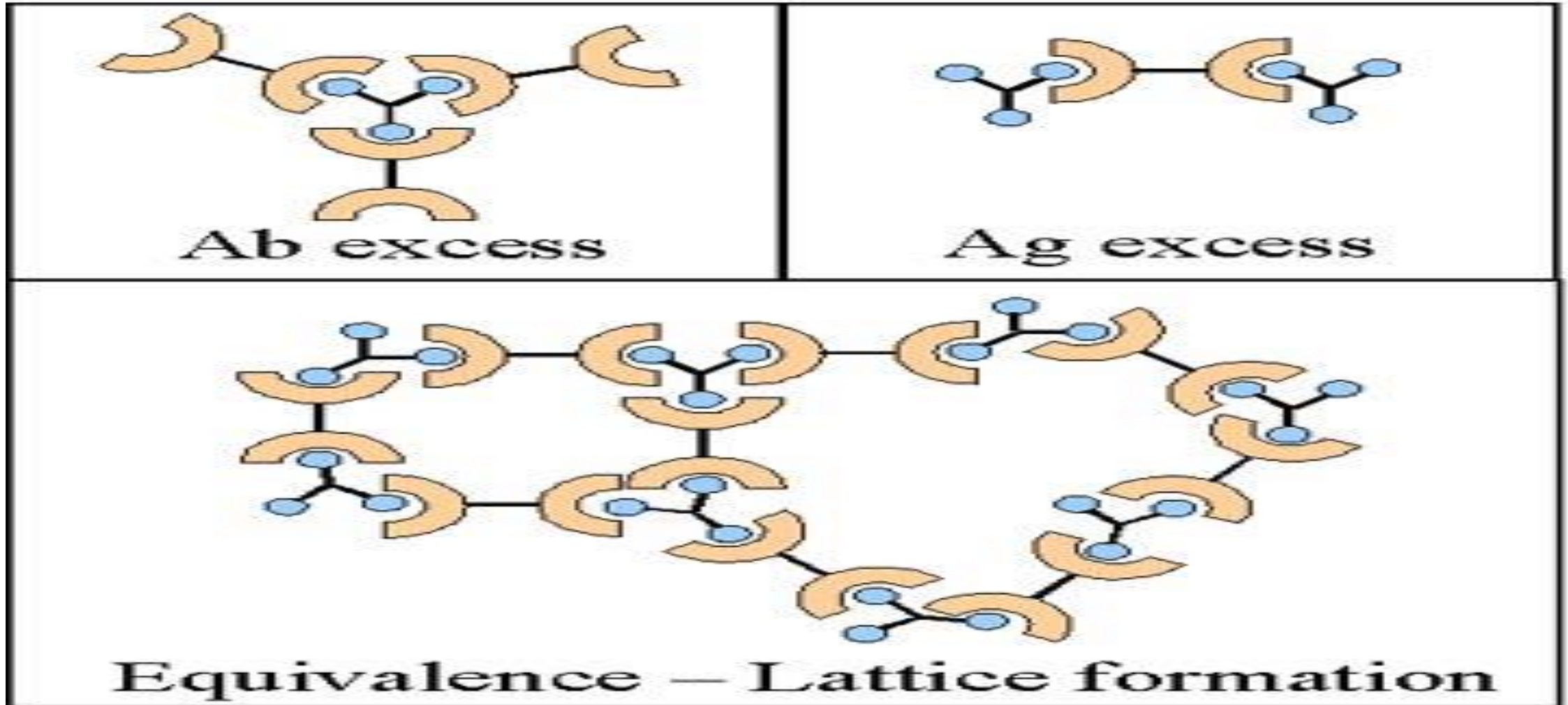
Applying the Law of Mass Action:

$$\mathbf{K_{eq} = \frac{[Ag-Ab]}{[Ag] \times [Ab]}}$$

Precipitation Curve

- Prozone – antibody excess, many antibodies coat all antigen sites- results in false negative
- Postzone – antigen excess, antibody coats antigen but cannot get lattice formation, results in false negative
- Zone of Equivalence – antigen and antibody present in optimal proportions to bind and give visible reaction

Precipitation Curve



Precipitation Curve

