



Microbiology/ 3<sup>rd</sup> Year M.B.CH.B. Students  
Part V: Basic & Clinical Immunology (17 hours)  
Lecture 8  
Duration: 1 hour

# Ag-Ab interactions

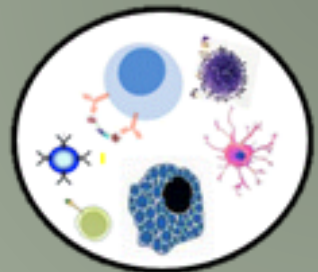
Assist. Prof. Dr. Nibras S. Al-Ammar



Reference: Immunology-a Short Course-Wiley-Blackwell (2015), Chapter 5, Page 67



For more detailed instruction, any question, cases need help please post to the group of session.



# Key Definitions



**Univalent Ab:** an incomplete form of antibody that may coat Ag, but does not have a second receptor for attachment to another molecule of Ag. Thus univalent Ab can not agglutinate Ags.

**Multivalent:** Ab have more than antigen-binding site.

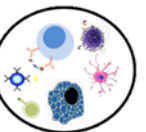
**Unideterminant Ag:** have one single type antigenic determinant (epitope), (e.g., Ag have only epitope A).

**Multideterminant Ag:** Have more than one type of antigenic determinant (epitope) (e.g., epitopes A, B &C).

**Univalent Ag:** have one binding site with the Ab because it have only single epitope.

**Multivalent Ag:** have more than one binding site with Ab (either the Ag have many epitopes but one type or the Ag have many epitopes and different types (e.g., epitopes A, B &C).

**Hydrogen bond:** interaction involving a hydrogen atom located between a pair of other atoms having a high affinity for electrons. It is weaker than an ionic bond or covalent bond but stronger than van der Waals forces.





**Electrostatic forces: (Coulomb force)** is the attractive or repulsive force between two electrically charged objects

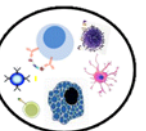
**Ionic bond:** is a type of chemical bond that involves the electrostatic attraction between oppositely charged ions, or between two atoms with sharply different electronegativity.

**Hydrophobic molecule:** nonpolar molecule that repelled from water (do not dissolve in water) and prefer other neutral molecules and nonpolar solvents (because water molecules are polar).

**Van der Waals forces:** week electric forces that attract neutral molecules to one another.

**Cross reaction:** Ag bind with an Ab that was raised to a different Ag. It occurs due to shared epitopes on multivalent Ags, or conformational similarity of epitopes.

**Titer:** highest dilution factor that still yields a positive reading (e.g., causes agglutination).





**Agglutination:** is the process of clumping of Ag particles by their respective Abs.

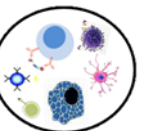
**Direct agglutination:** Ag is a natural constituent of a particle.

**Passive agglutination:** when the agglutination reaction takes place between Abs & soluble Ag that had been attached to insoluble particle (latex).

**Precipitation:** is a process where soluble Ags bind with their specific Abs at an optimum temperature & pH, resulting in the formation of an insoluble precipitate.

**Lattice formation:** cross-linkages formation.

**Titer:** highest dilution factor that still yields a positive reading (e.g., causes agglutination).



# Learning objectives (LOs)



**Introduction about Ag-Ab interactions**

**LO.1**

**Affinity & Avidity**

**LO.2**

**Primary interactions between Ag &Ab**

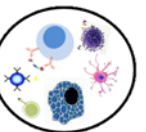
**LO.3**

**Secondary interactions between Ag &Ab**

**LO.4**

**Immunoassays**

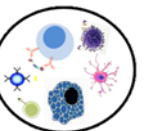
**LO.5**





# Introduction about Ag-Ab interactions

- **Antigen-Antibody interaction occurs by combining Ag with Ab specifically.**
- **It forms the basis for humoral immunity (antibody mediated immunity).**
- **These interactions are used for detection of infectious agents and some non-specific Ag like enzymes.**



# Affinity & Avidity

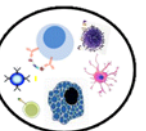
LO.2



**Affinity:** The strength of an interaction between a single binding site on an Ab & its target epitope.

**Avidity:** The measure of the total binding strength of an Ab at every binding site. It also known (the functional affinity).

**Note:** IgM have higher avidity than IgG, although the binding of each Fab in IgM with antigenic determinant may be the same affinity as that of the Fab from IgG.



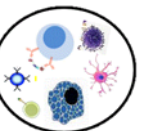


## Primary interactions between Ag & Ab

- No covalent bonds involved
- Binding forces are relatively weak, consist mainly of:
  - a. van der Waals forces
  - b. hydrophobic forces
  - c. electrostatic forces

\*Ag-Ab interaction requires a very close fit between an epitope & the Ab (like a lock & key).

\*Ag-Ab complexes readily dissociated by low or high pH, high salt concentrations.





# Reactions between Ab or Ab fragments & Ag or hapten

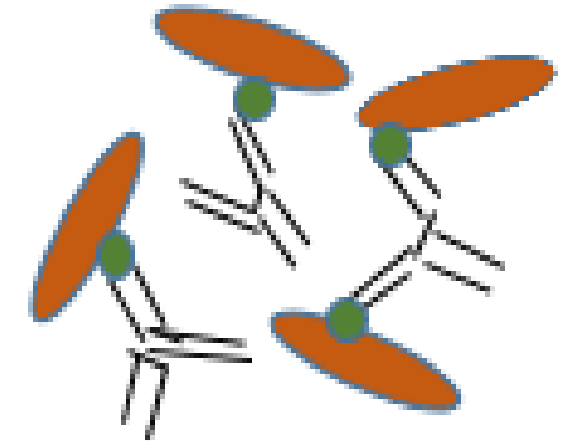
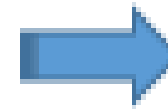
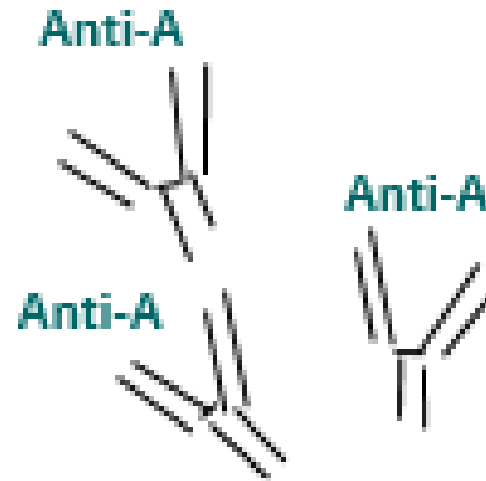
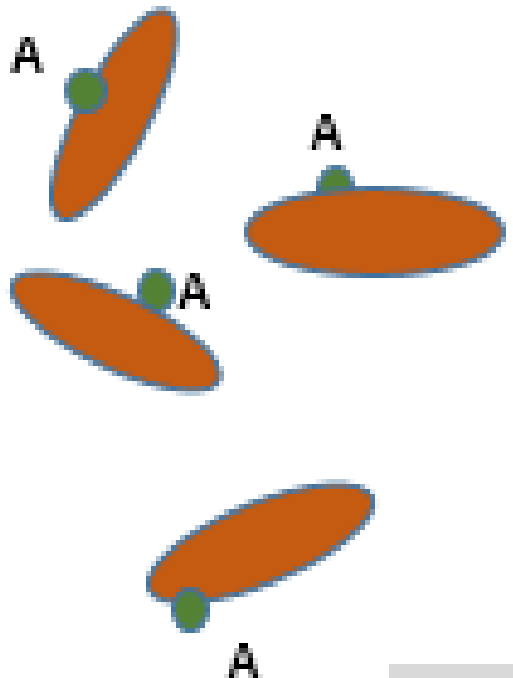
LO.3



Univalent, Unideterminant  
Ag (hapten)

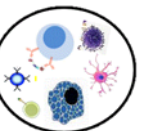
Anti-A

(A-Anti-A) Complexes  
Not cross-linked



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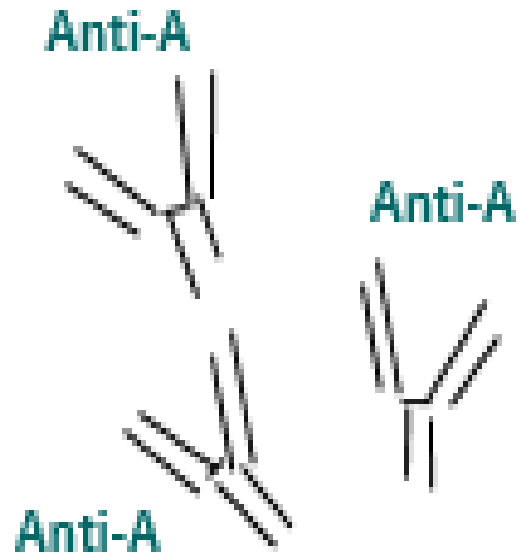
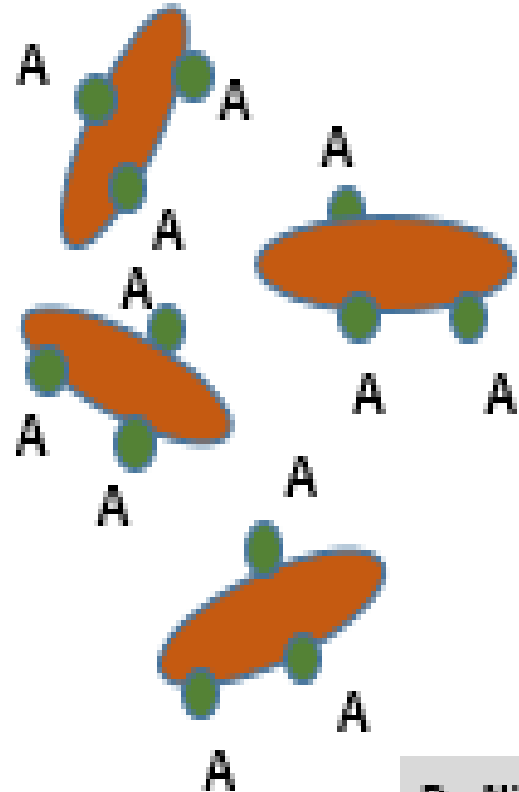
A





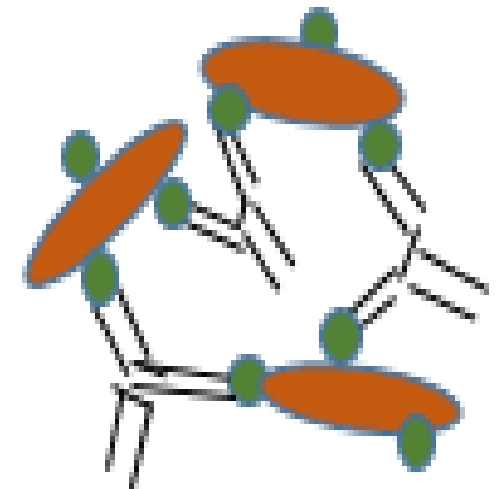
Multivalent, Unideterminant

Ag



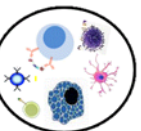
(A-Anti-A), Complexes

Cross-linked



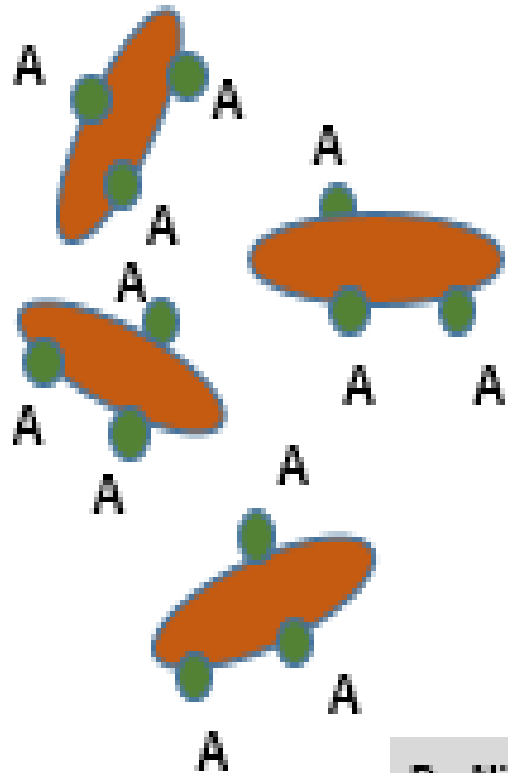
**B**

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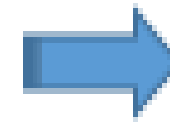
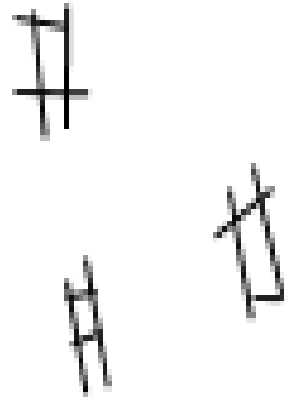




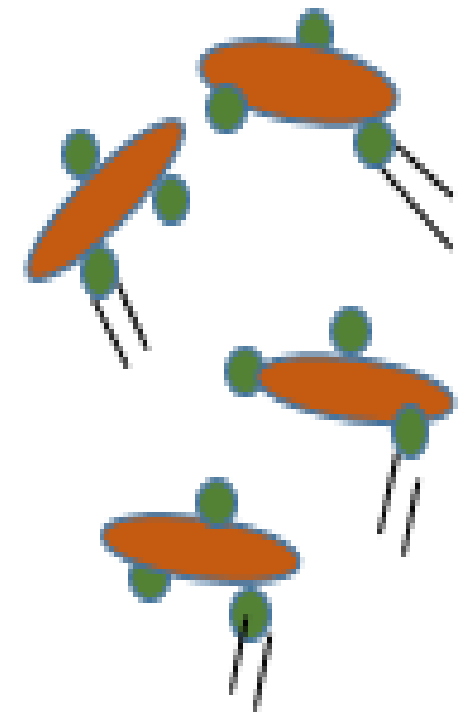
Multivalent, Unideterminant  
Ag



Anti-A  
Fab

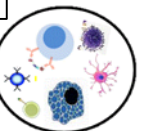


(A-Anti-A Fab) Complexes  
Not cross-linked



C

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Multivalent, Unideterminant

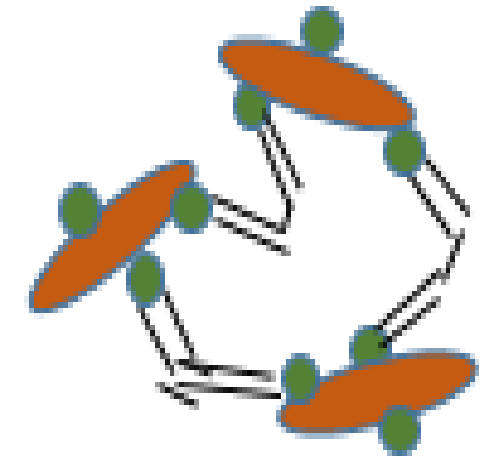
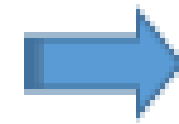
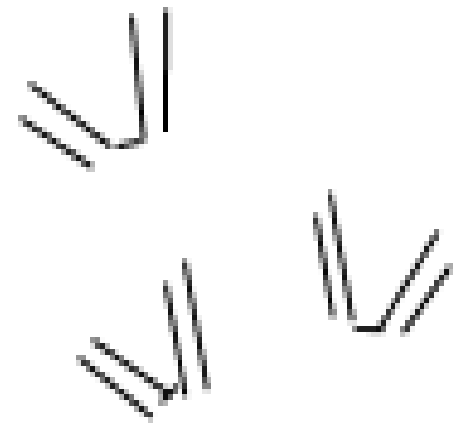
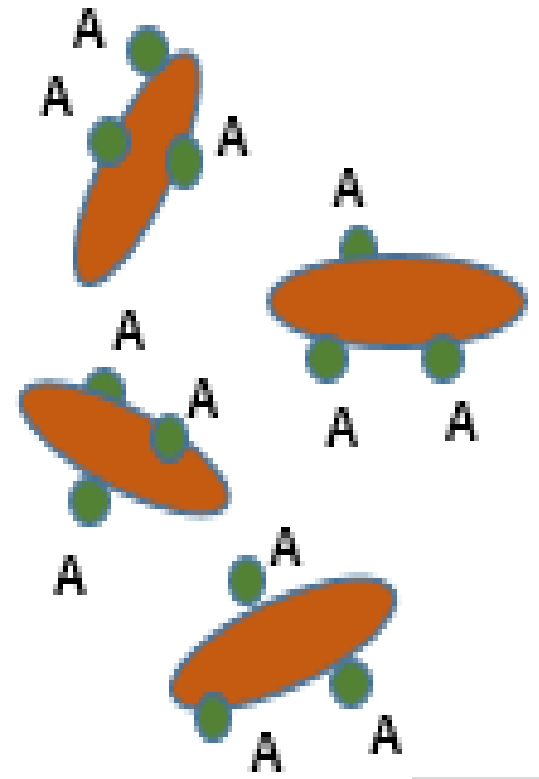
Ag

Anti-A (Fab)<sub>2</sub>

A-Anti-A (Fab)<sub>2</sub>

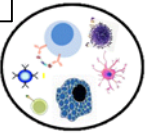
Complexes

cross-linked



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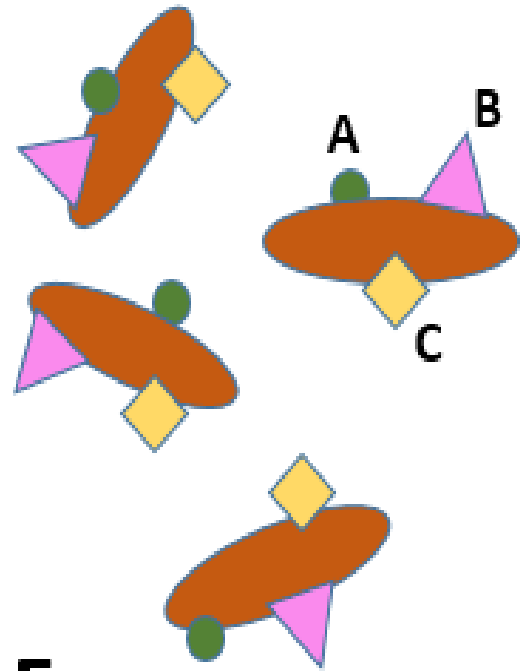
D



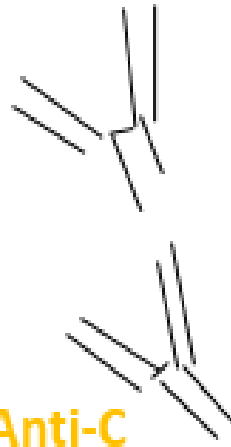


Multivalent, Multideterminant

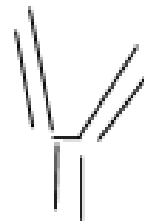
Ag



Anti-A



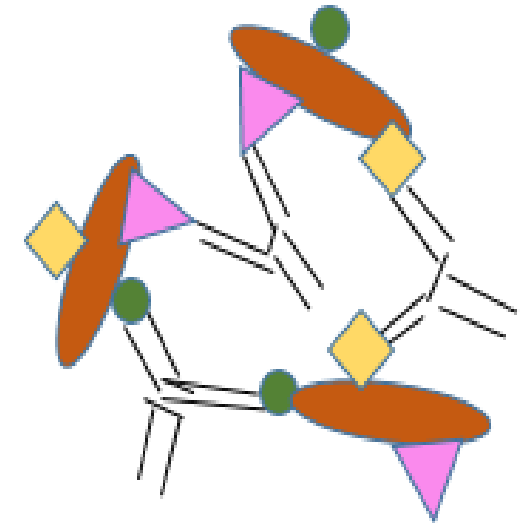
Anti-B



Anti-C

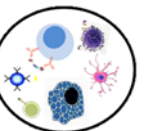


(A-Anti-A), (B-Anti-B), (C-Anti-C)  
Complexes



E

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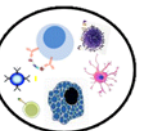


## Secondary interactions between Ag & Ab (Lattice formation)

**Different outcomes may result from secondary Ag-Ab interactions:**

- **Agglutination (Ag is particulate)**
- **Precipitation (Ag is soluble)**
- **Activation of complement**

**All these outcomes caused by interactions between multivalent Ags & Abs that have at least 2 combining sites per molecule**

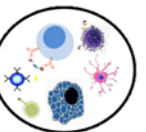




# Agglutination reactions

- Semi-quantitative.
- Performed by mixing twofold serial dilutions of serum (Ab) with a fixed concentration of Ag.
- Agglutination may not occur when Abs present in excess (Prozone phenomenon).
- Agglutination may not occur when Ags present in excess (Postzone phenomenon).

**Q.1: Antiserum tested at several dilutions. Why?**

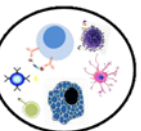


# Zeta potential



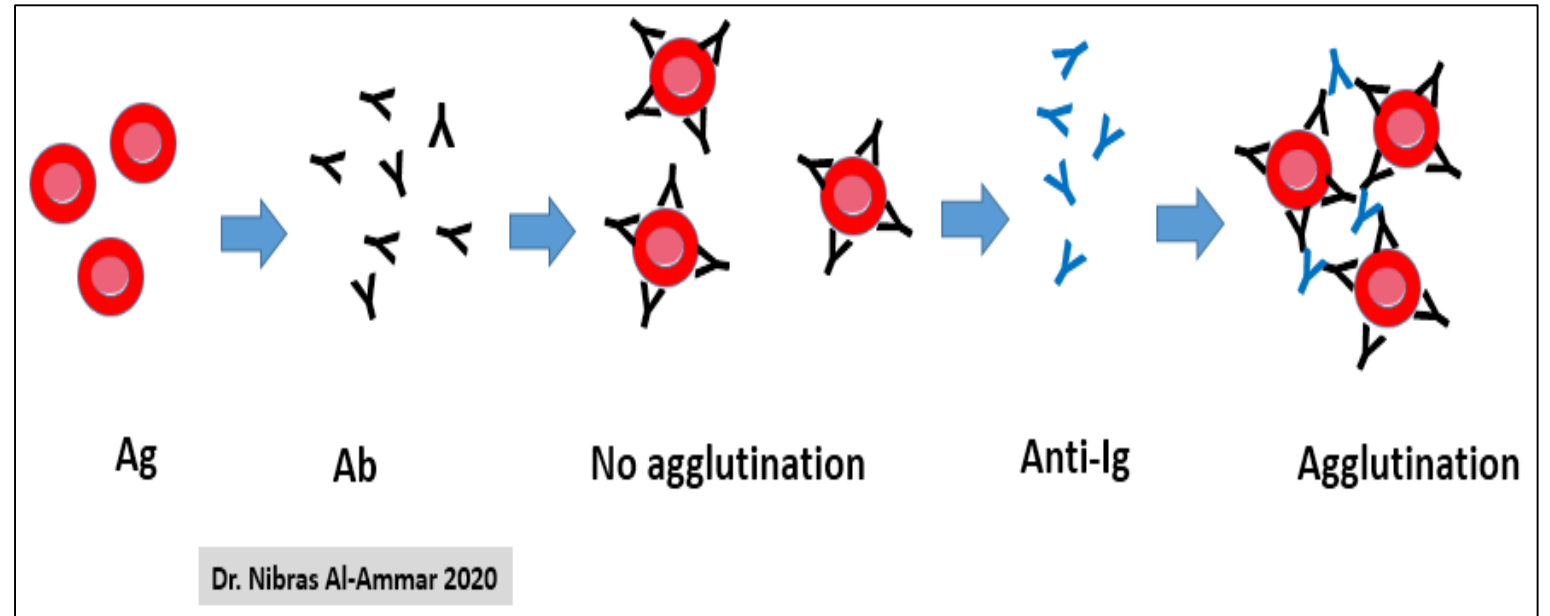
- The surfaces of certain particulate Ags may possess an electrical charge (e.g., the net negative charge on the surface of RBCs) caused by the presence of sialic acid.
- When such charged particles suspended in saline solution, an electrical potential created between particles known as (**Zeta potential**), preventing them from getting close to each other.
- **Zeta potential** makes it difficult to agglutinate charged particles by Abs.

**Q.** Regarding to Zeta potential, which is more effective as agglutinating Abs, IgG or IgM?



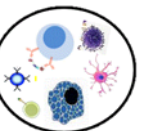


In 1950s Coombs created a method to overcome **Zeta potential** by employing Abs to Igs (**anti-Ig**).



### Examples of agglutination reactions tests:

- **Direct Coombs** – hemolytic disease of newborn (HDN)
- **Indirect Coombs** – Detection of anti-Rh IgG Abs in the blood of an Rh- woman.
- **Blood grouping**
- **Pregnancy test**



# Precipitation Reaction

(LO.4)



## 1. Precipitation reactions in liquids (e.g., Widal test for testing infection with *Salmonella typhi*):

**1. Adding a constant amount of Ab in each tube**

**2. Adding increased amount of Ag in each tube**

**3. Ag-Ab interact & precipitate in the bottom of the tube**

**4. Plotting the amount of precipitate against increasing antigen concentration yields a precipitation curve.**

The diagram illustrates the precipitation reaction in three zones: Ab excess zone (yellow), Equivalence zone (green), and Ag excess zone (orange). Above the zones are illustrations of antibody-antigen interactions. Below the zones are ten test tubes showing the amount of precipitate formed. The fifth tube, representing the equivalence zone, is circled in red. A large blue arrow points from left to right across the tubes.

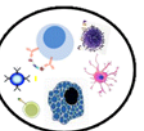
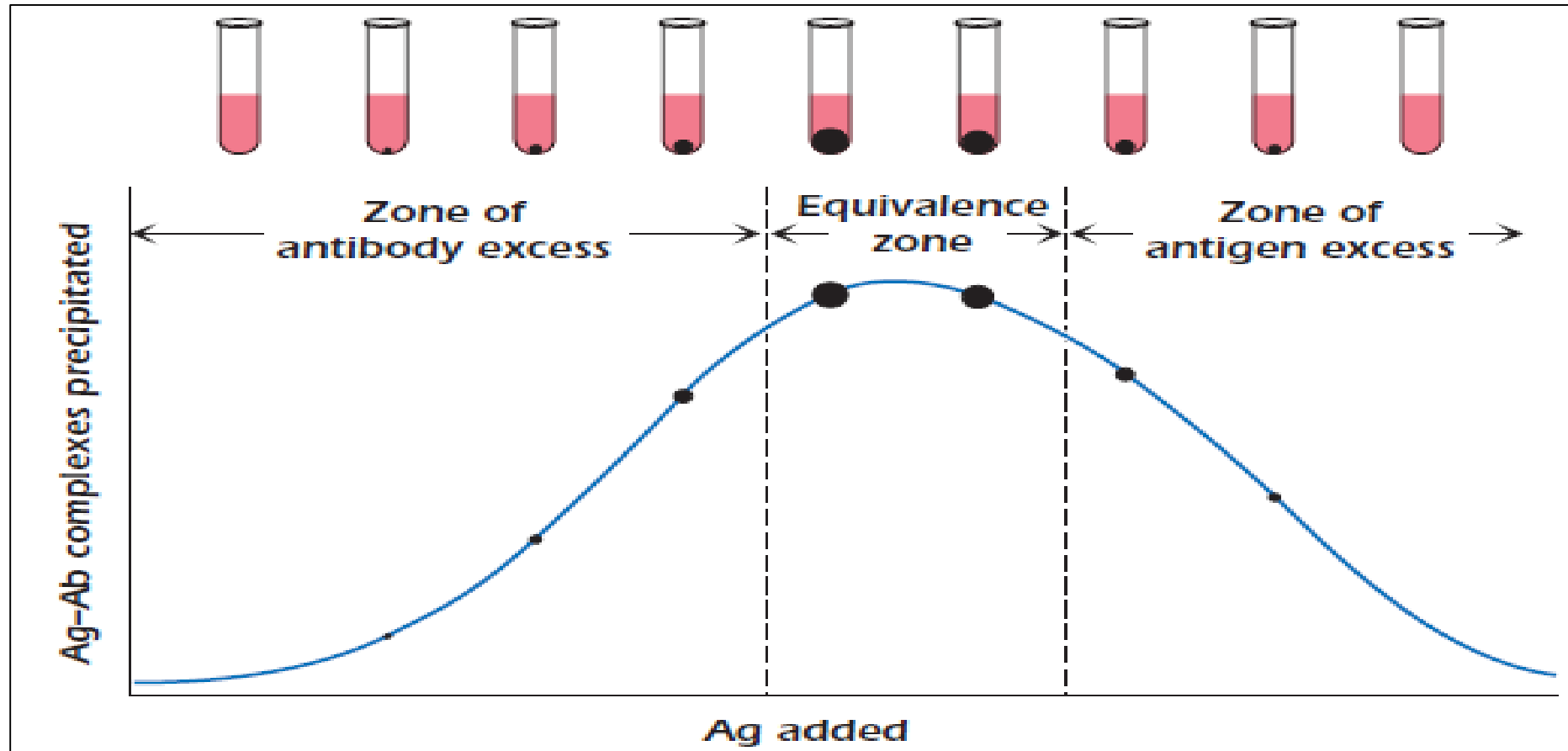
The graph plots the amount of precipitate (y-axis, 0 to 1.0) against increasing antigen concentration (x-axis, 0 to 400). The curve rises to a peak at an antigen concentration of 100, labeled as the Equivalence point, and then gradually declines.

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# Precipitation curve:



When increasing concentrations of Ag added to a series of tubes contain a constant concentration of Abs, variable amounts of precipitate form. Precipitin curve can be obtained by blotting the amount of precipitate against the amount of Ag added.

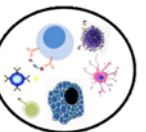




LO.4

## 2. Precipitation reactions in gel: Some examples:

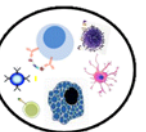
- Radial Immunodiffusion
- Immunoelectrophoresis
- Western Blot (Immunoblot)





# Immunoassays

- **Direct-binding Immunoassays: (RIA)**
- **Solid-phase Immunoassays: (ELISA)**
- **Immunofluorescence**
- **Flow Cytometry**





*Thank You*

