University of Basrah, Medical College – Microbiology Department

Microbiology/ 3rd Year M.B.CH.B. Students

Part V: Basic & Clinical Immunology (17 hours)

Lecture 8

Duration: 1 hour

Antigen-Antibody interactions

Assist. Prof. Dr. Nibras Saleam 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 an antibody that may coat Ag, but does not have a second receptor for attachment to another molecule of Ag. Univalent Ab cannot agglutinate Ags.

Multivalent: Ab have more than antigen-binding site.

Unideterminant Ag: have 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 Ab because it have only single epitope.

Multivalent Ag: have more than one binding site with Ab (either 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 id 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 articles 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

(LO.1)

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 used for detection of infectious agents and some non-specific Ag like enzymes.

(LO.2)

Affinity & Avidity

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.

(LO.3)

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

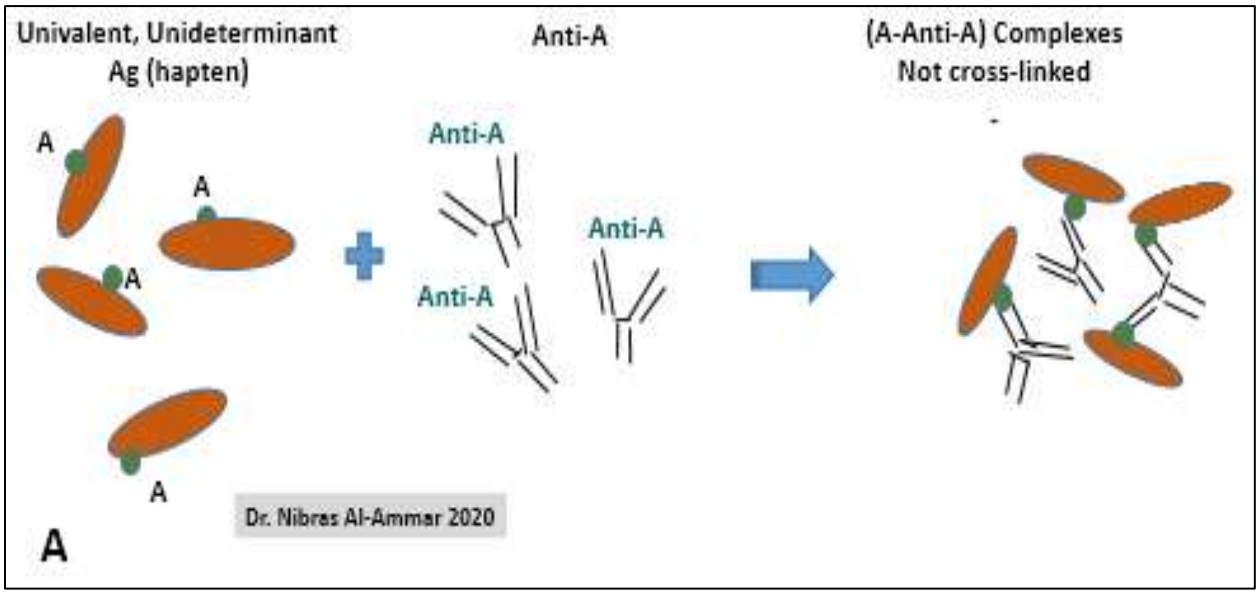
c. electrostatic forces

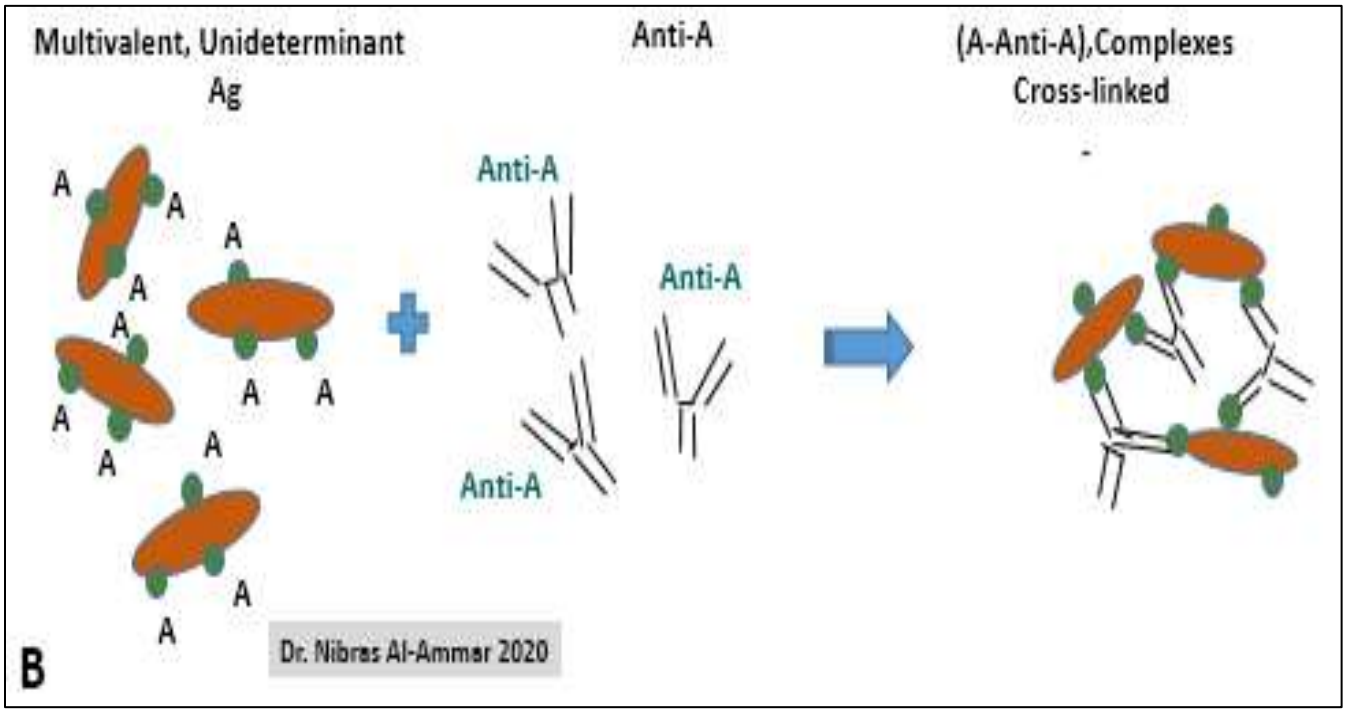
***Ag-Ab interaction requires a very close fit between an epitope & the Ab (like a lock & key).**

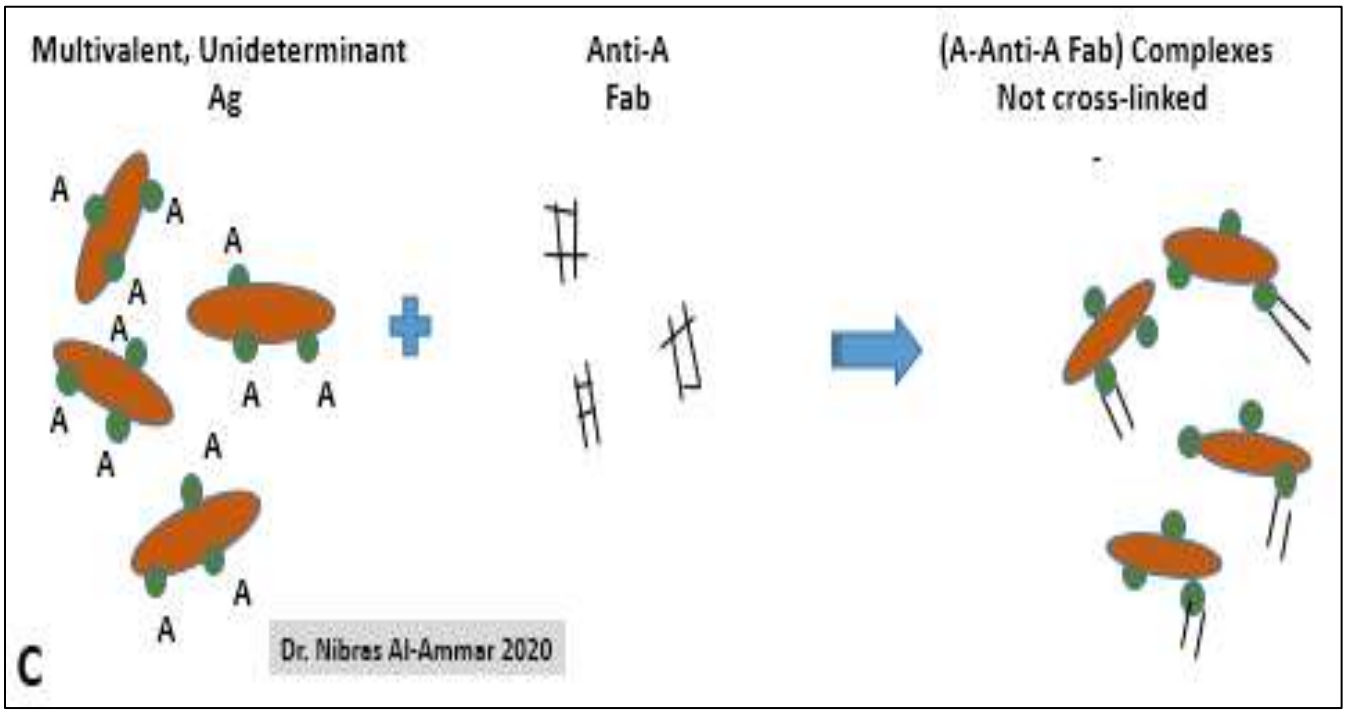
***Ag-Ab complexes readily dissociated by low or high pH, high salt concentrations.**

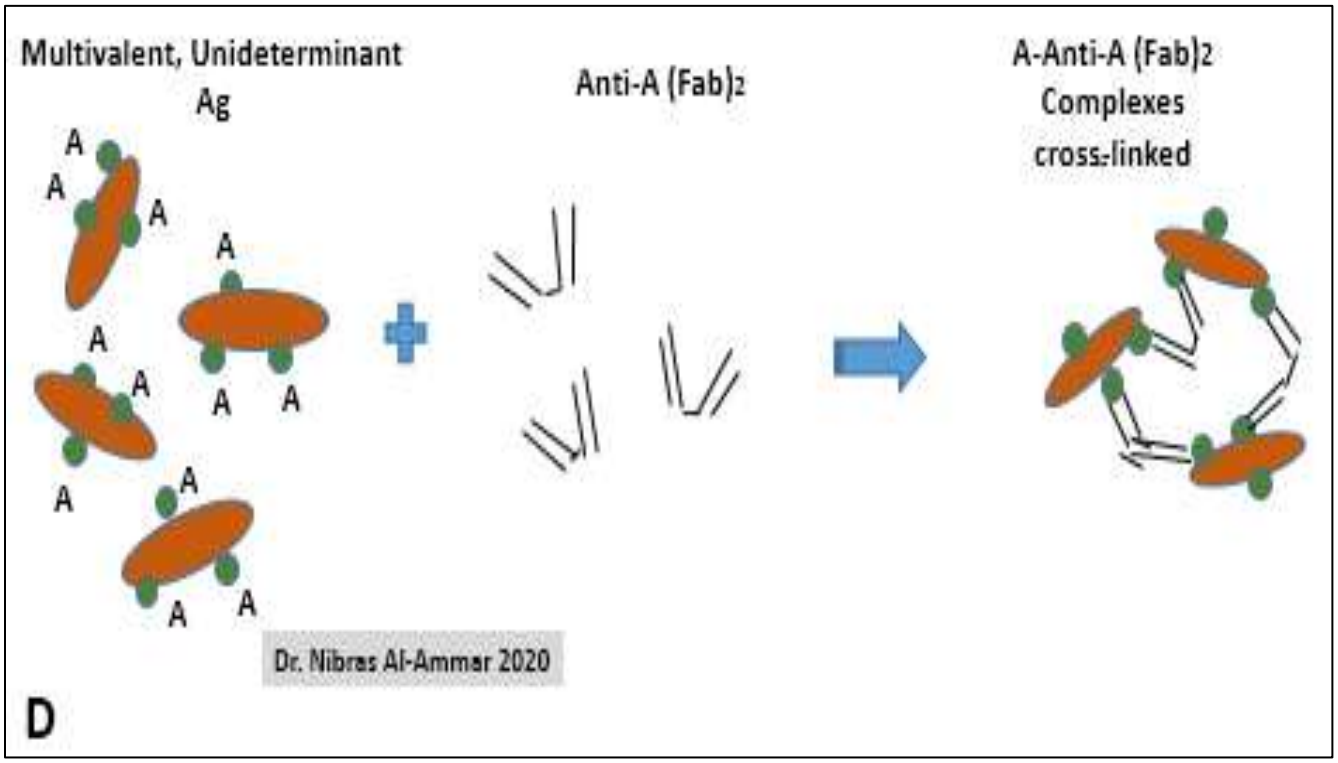
(LO.3)

Reactions between Ab or Ab fragments & Ag or hapten




Multivalent, Unideterminant
Ag
Anti-A
(A-Anti-A),Complexes
Cross-linked
Anti-A
Anti-A
Anti-A
Dr. Nibras Al-Ammar 2020
B


Multivalent, Unideterminant
Ag
Anti-A
Fab
(A-Anti-A Fab) Complexes
Not cross-linked
Dr. Nibras Al-Ammar 2020
C


Multivalent, Unideterminant
Ag
Anti-A (Fab)2
A-Anti-A (Fab)2
Complexes
cross-linked
A
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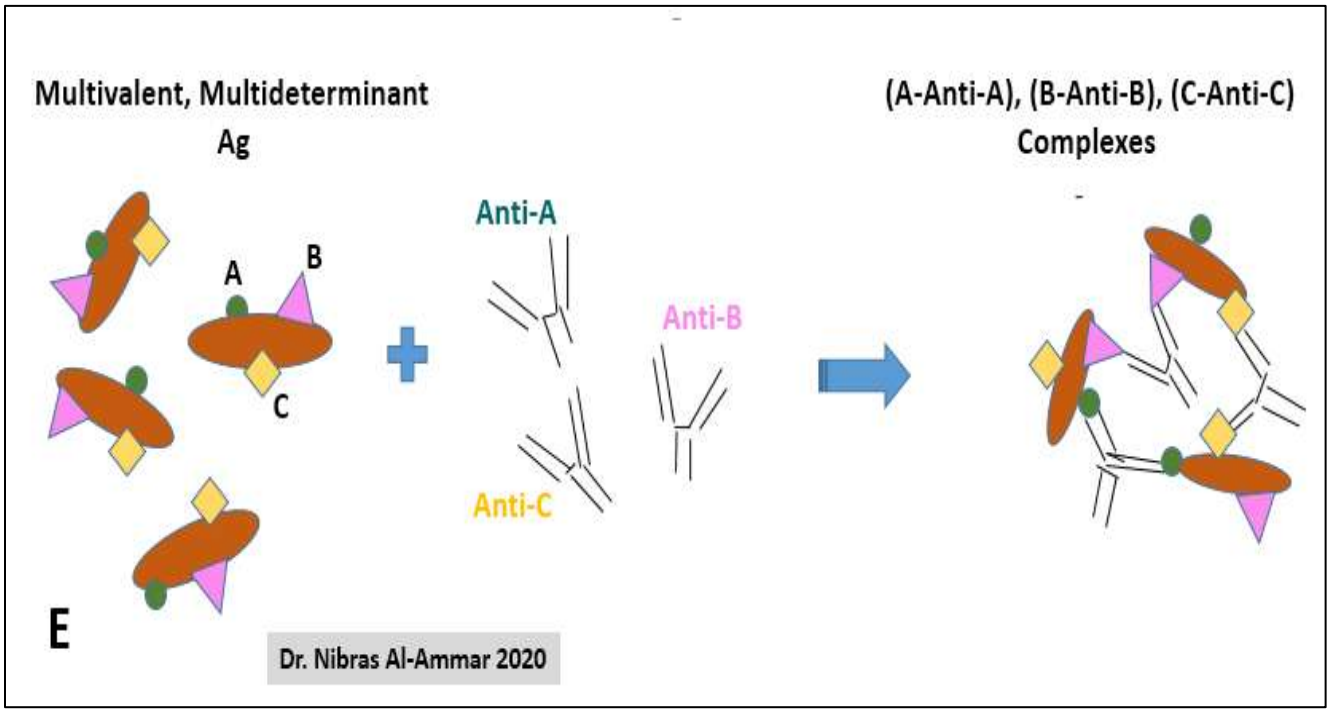
D


Multivalent, Multideterminant
Ag
(A-Anti-A), (B-Anti-B), (C-Anti-C)
Complexes
Anti-A
Anti-B
Anti-C
A
B
C
Dr. Nibras Al-Ammar 2020
E

LO.4

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**

LO.4

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.: Antiserum tested at several dilutions. Why?

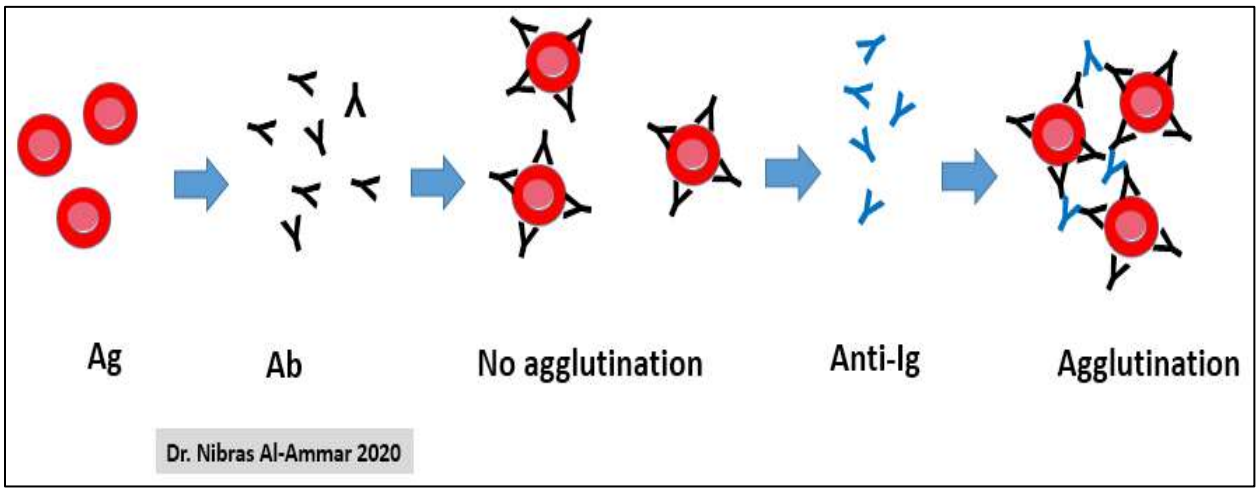
LO.4

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 know 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).



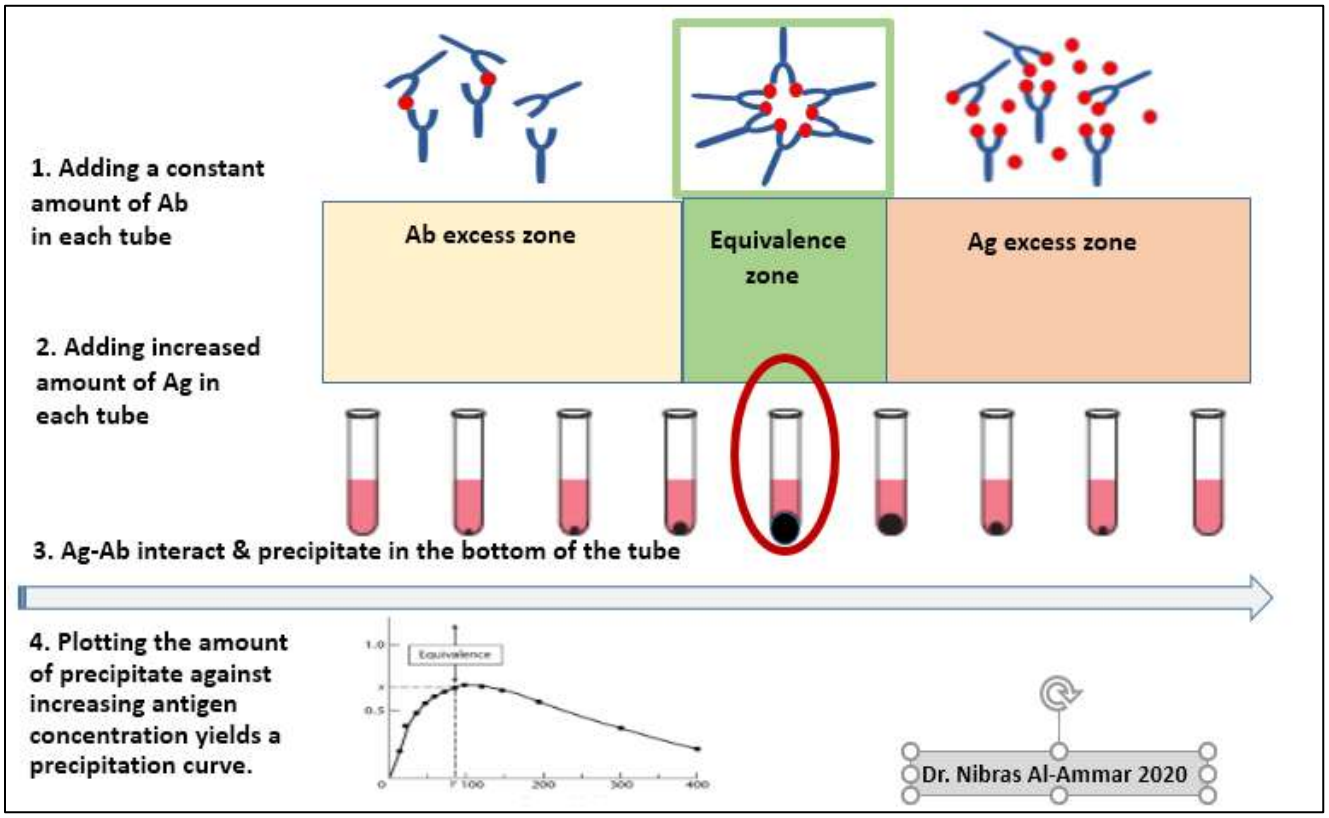
Examples of agglutination reactions tests:

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

LO.4

Precipitation Reaction

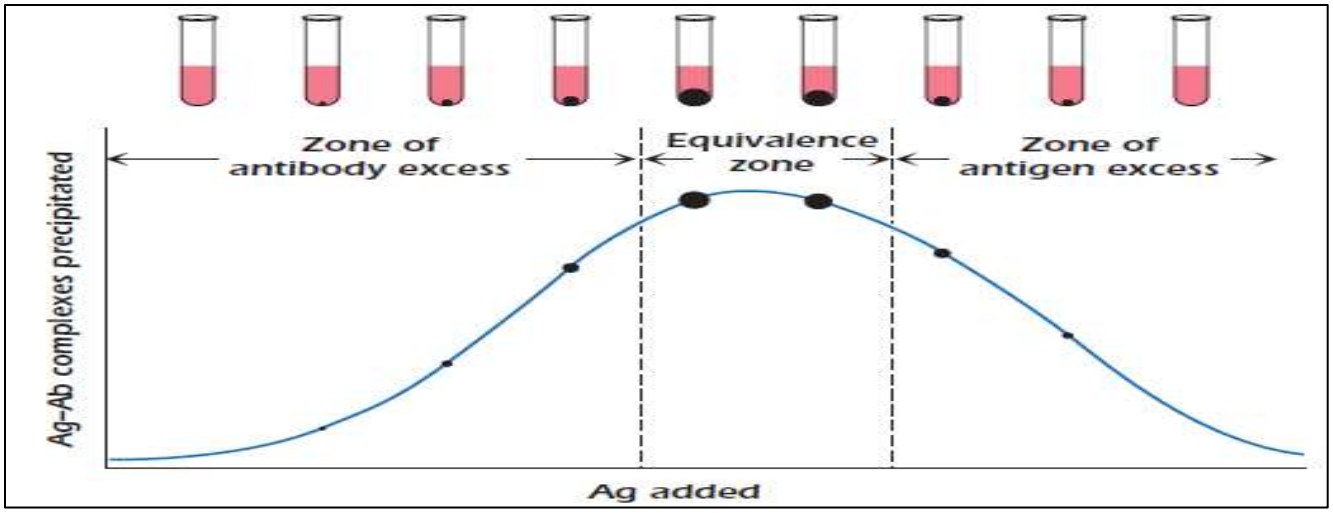
1. **Precipitation reactions in liquids (e.g., Widal test for testing infection with *Salmonella typhi*)**



LO.4

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 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)**

LO.5

Immunoassays

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

☺

Thank You