



Cihan University/ Sulaymaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 4: Immunoassay

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Immunoassay

- The combination of an antigen with a specific antibody plays an important role in the laboratory in diagnosing many different diseases.
- It is an analytical method which uses antibodies as reagents to quantitate specific analytes.
- Immunoassays have been developed to detect either antigen or antibody.
- The first such assays were based on the principles of precipitation or agglutination.
- Precipitation involves combining soluble antigen with soluble antibody to produce insoluble complexes that are visible.
- Agglutination is the process by which particulate antigens, such as cells, aggregate to form larger complexes when a specific antibody is present.
- **Precipitation and agglutination** are considered unlabeled assays because a **marker label is not needed to detect the reaction**.



Immunoassays

- Based on the specificity of the interaction between antigen and antibody.
- Antibody can be produced against any type of macromolecules.
- **Production of antiserum-** Antibodies used for **research and diagnostic purpose** are often obtained by injecting a lab animal such as rabbit or a goat with a **specific antigen**.

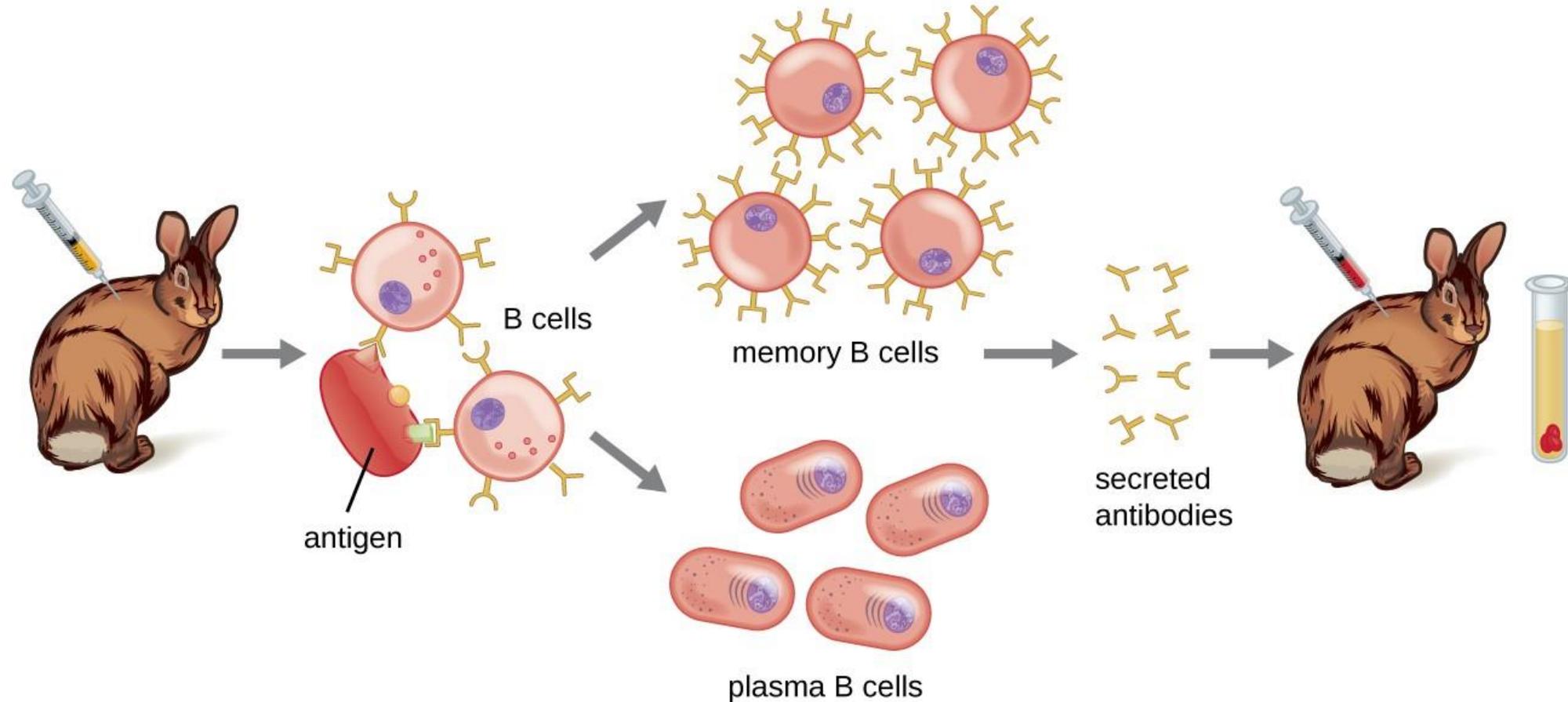
Production of Antiserum- Antibodies

1 Inject antigen into rabbit.

2 Antigen activates B cells.

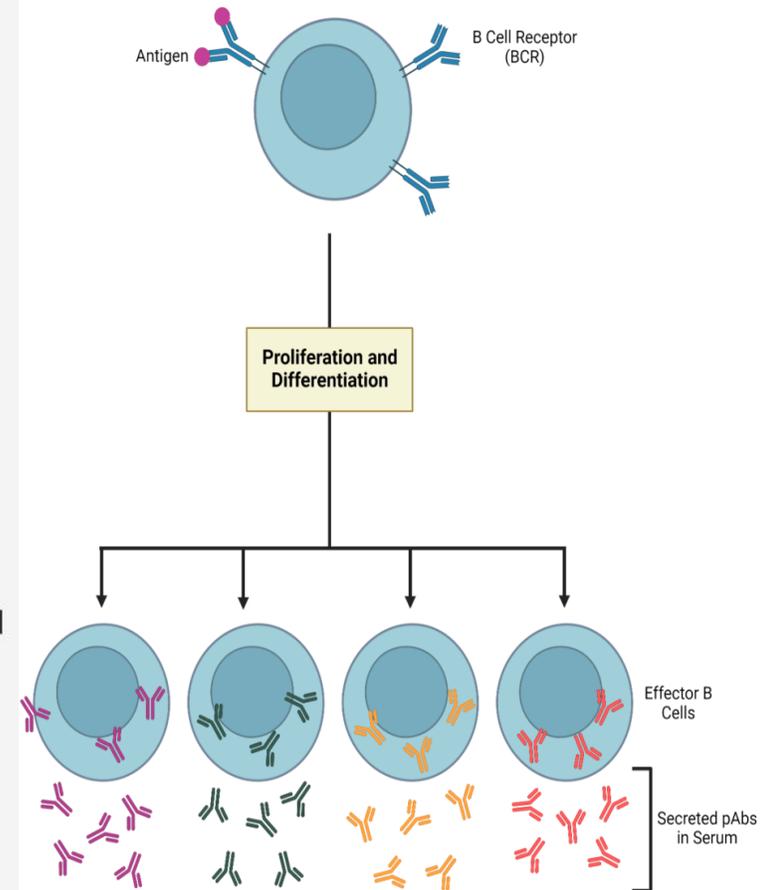
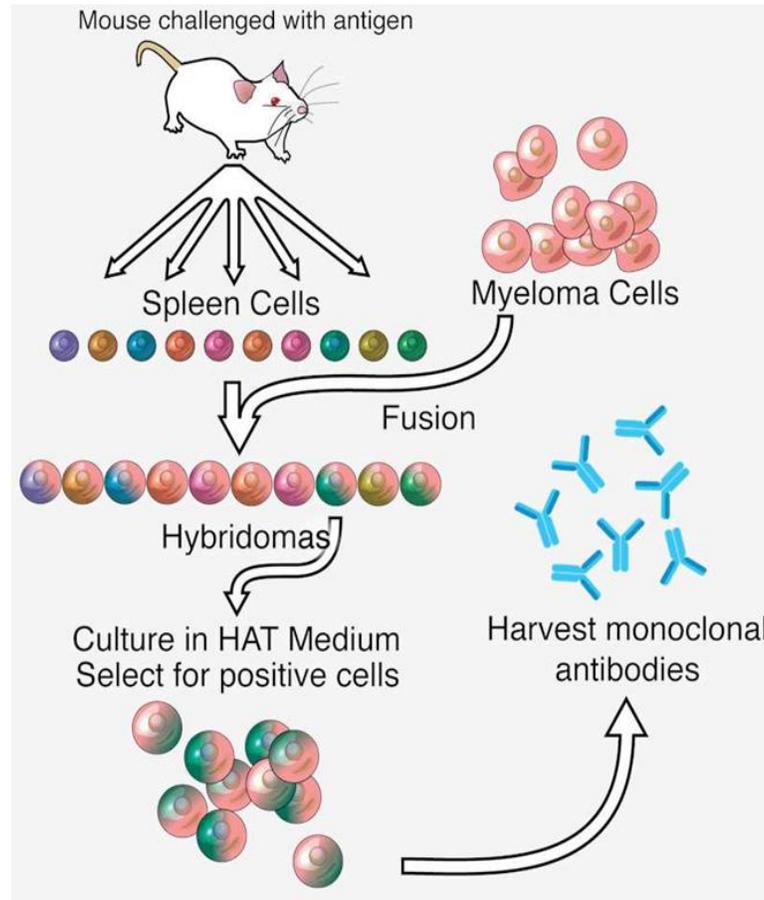
3 Plasma B cells produce polyclonal antibodies.

4 Obtain antiserum from rabbit containing polyclonal antibodies.



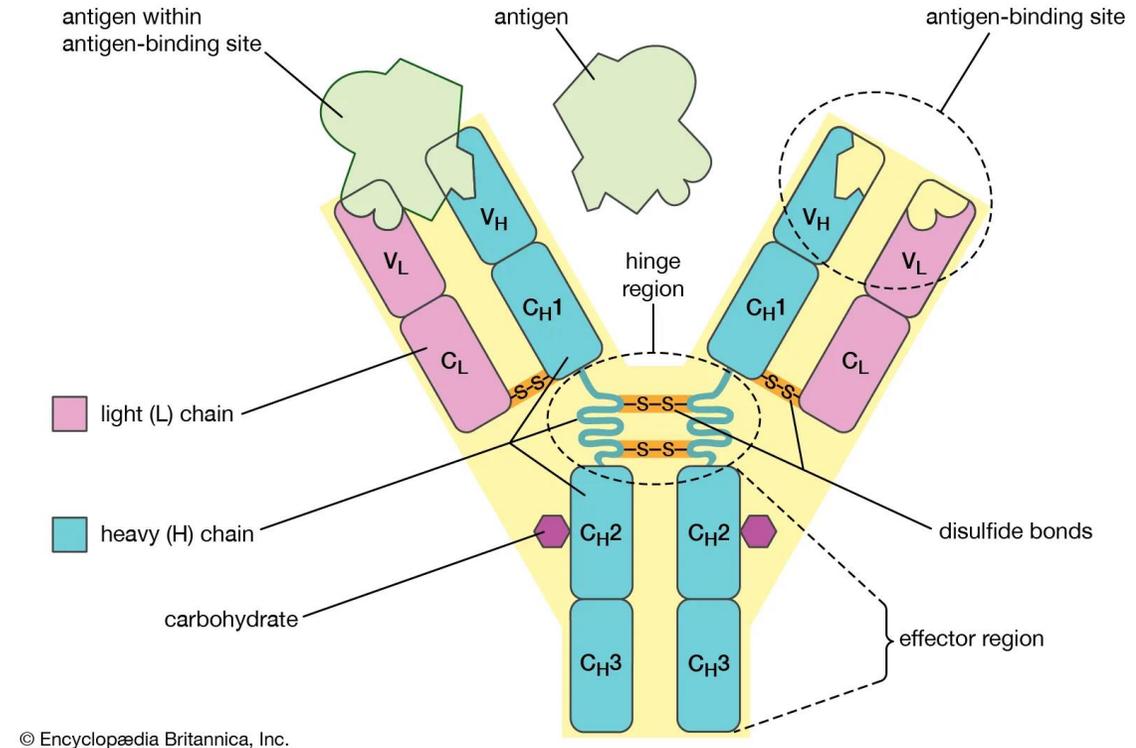
Types of antibodies

- **Polyclonal antibodies:** recognize multiple sites on antigens.
- **Monoclonal antibodies;** recognize single site on antigen.



Antigen–Antibody Binding

- The primary union of binding sites on an antibody with specific epitopes on an antigen depends on two characteristics of antibody known as **affinity** and **avidity**.
- These characteristics are important because they relate to the sensitivity and specificity of testing in the clinical laboratory.



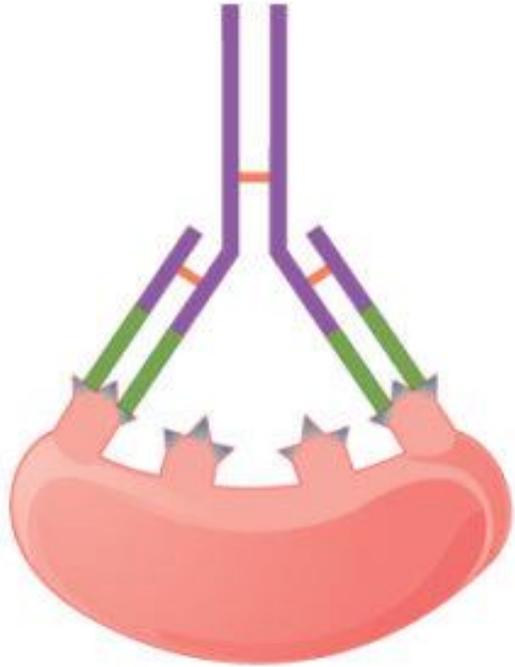


Affinity, Avidity and Cross Reactivity

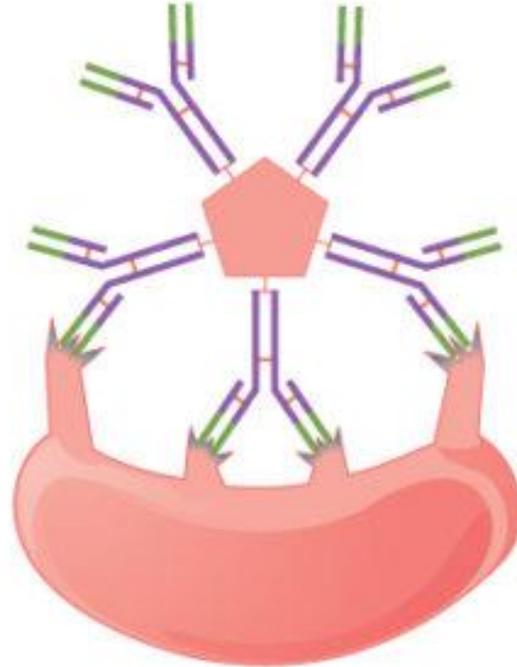
- ❖ **Affinity:** is the initial force of attraction that exists between a single Fab site on an antibody molecule and a single epitope on the corresponding antigen. The strength of attraction depends on the specificity of antibody for a particular antigen.
- ❖ **Avidity:** represents the overall strength of antigen–antibody binding and is the sum of the affinities of all the individual antibody–antigen combining sites.
 - Immunoglobulin M (IgM), for instance, has a higher avidity than IgG because IgM has the potential to bind 10 different antigens.
- ❖ **Cross Reactivity:** the capacity of a single antibody to recognize the same or structurally similar epitopes on different antigens.

Affinity, Avidity and Cross Reactivity

(a) Affinity versus avidity

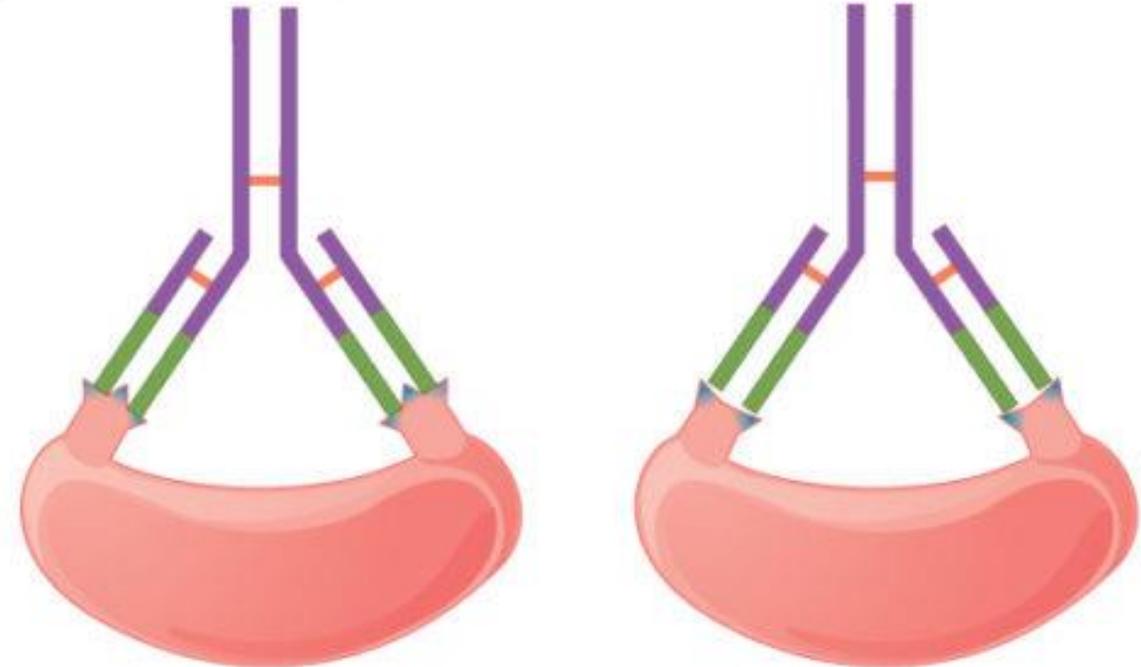


Affinity refers to the strength of a single antibody–antigen interaction. Each IgG antigen binding site typically has high affinity for its target.



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.

(b) Cross reactivity



An antibody may react with two different epitopes.



Application of Serological Tests

It is widely used for diagnostics purposes in the medical laboratory.

1. Detection of **serum antibodies** to **specific infectious agent**.
2. Identification **antigen** in clinical specimen.
3. Protein identification.

Precipitation Techniques

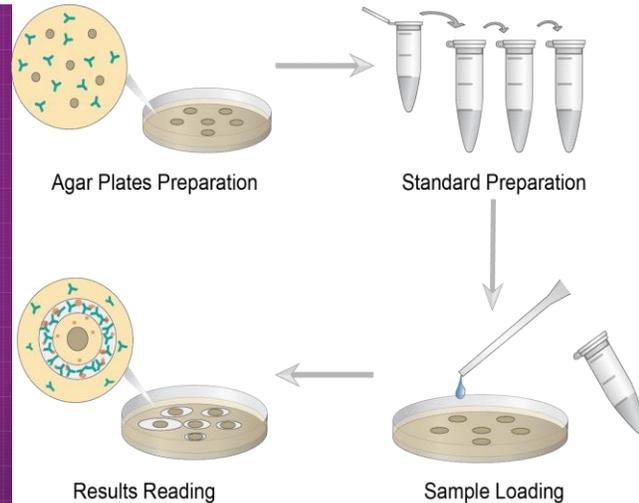
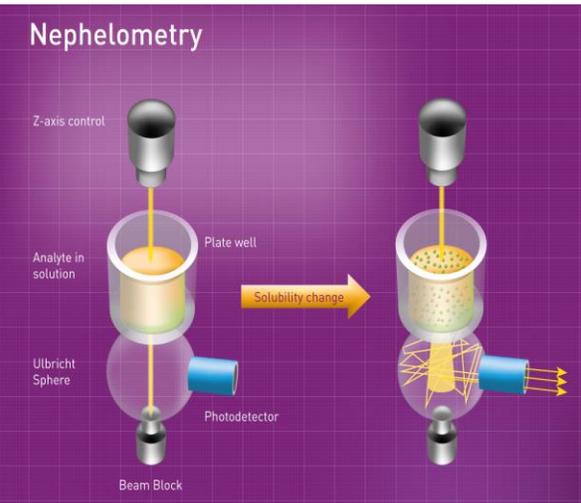
Nephelometry

Radial Immunodiffusion (RID)

Ouchterlony double diffusion

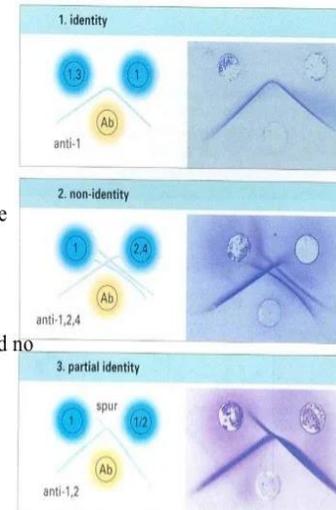
Immunofixation electrophoresis (IFE)

Nephelometry

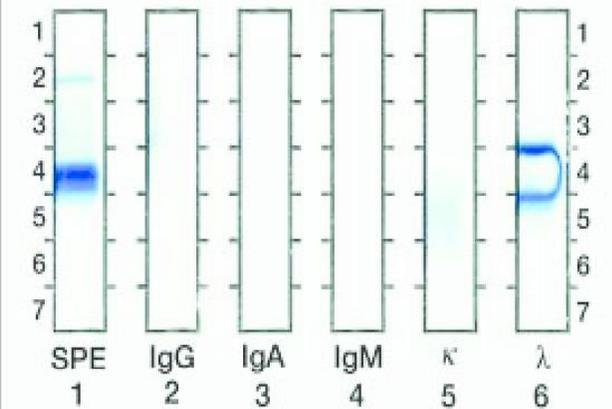


Diffusion patterns

- **Fusion of lines at their junction to form an arc**
 - Serologic identity / presence of common epitope
- **Crossed lines**
 - Demonstrates 2 separate reactions
 - Compared antigens shared no common epitopes
- **Fusion of 2 lines with spur**
 - Partial identity



BECKMAN Paragon IFE Gel



Agglutination Reactions

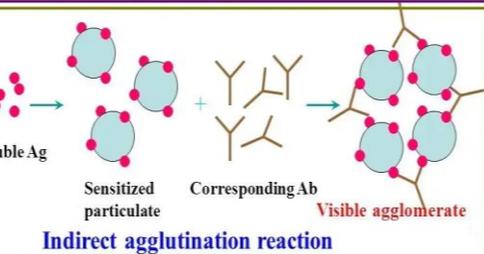
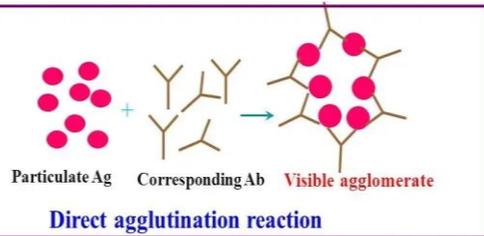
Direct agglutination

Indirect (passive) agglutination

Reverse passive

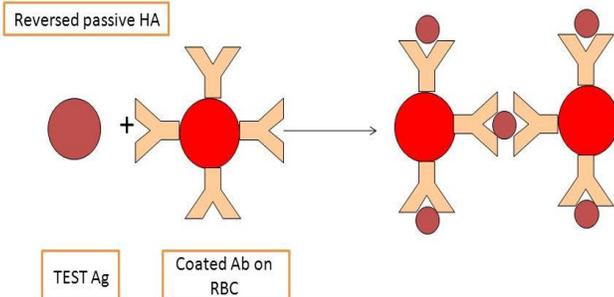
Agglutination inhibition

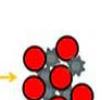
Hemagglutination inhibition

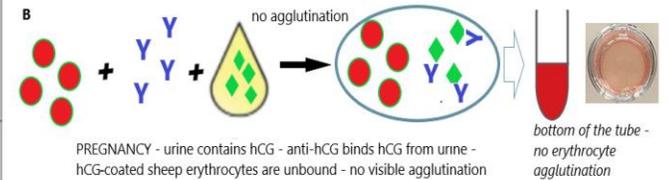
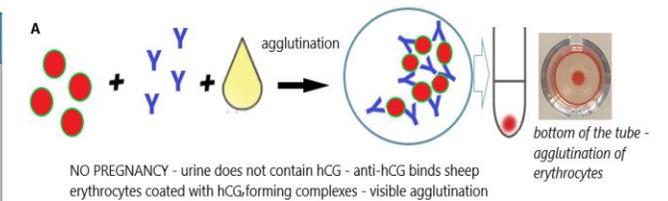


Reversed passive haemagglutination

- It is an agglutination where Ab is coated on the red blood cell.



Components	Interaction	Microtiter Results
RBCs		No Reaction
Virus + RBCs		Hemagglutination
Virus + Antibody + RBCs		Hemagglutination Inhibition



Legend:  hCG  anti-hCG (animal)  urine tested  Sheep erythrocytes coated with hCG



References

- Abbas, A. K., Lichtman, A. H., Pillai, S., & Baker, D. L. (. i. (2020). Basic immunology: Functions and disorders of the immune system (Sixth edition.).
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