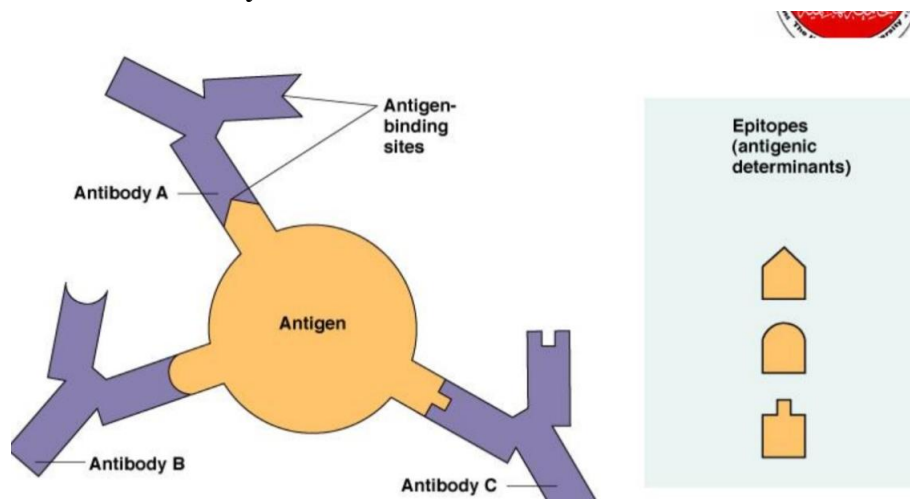


# Definitions

- **Antigen**: is any substance that causes your immune system to prompt the generation of antibodies
- **Antibodies**: Immunoglobulins that recognize and bind to a particular antigen with high specificity and is made in response to exposure to the antigen
- **Epitope**: Small part of an antigen that interacts with an antibody (10-12 amino acids). Any given antigen may have several epitopes. Each epitope is recognized by a different antibody



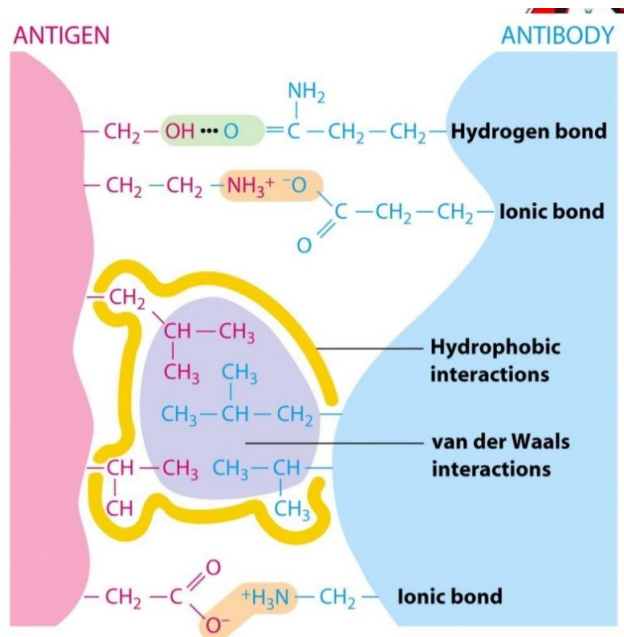
## Antibody-Antigen Interaction

- The interaction of the antibody with an antigen causes a **change in shape** of the antibody
- May cause the exposure of another site which then is responsible for the various reactions elicited by the antibody to destroy the foreign substance.
- The interaction of antibodies and antigens may produce a **network type complex**

## Nature of Antigen Antibody Reaction

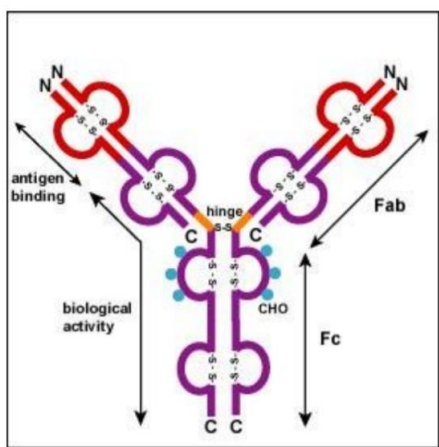
- **Lock and Key Concept**: The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains
- **Non-covalent Bonds**: The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include **hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds.**
- **Reversibility**: Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible

The Ag-Ab interaction is due to lots of non-covalent interactions lock and key!



### Antigen-antibody binding site

- The Fab portion of the antibody has the complementarity-determining regions (red) providing specificity for binding an epitope of an antigen.
- The Fc portion (purple) directs the biological activity of the antibody.
- (S-S = disulfide bond; N = amino terminal of glycoprotein; C = carboxy terminal of glycoprotein; CHO = carbohydrate.)

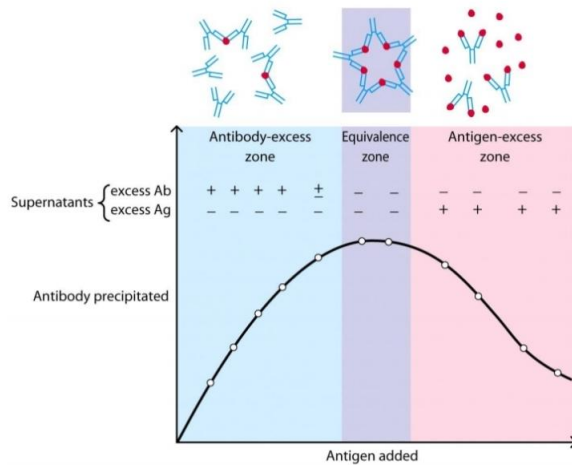


### Antibody Binding Variations

- The various genes the cell splices together determine the order of amino acids of the Fab portion of both the light and heavy chain; the amino acid sequence determines the final 3-dimensional shape.
- Therefore, different antibody molecules produced by different B-lymphocytes will have different orders of amino acids at the tips of the Fab to give them unique shapes for binding epitope.

- The antigen-binding site is large enough to hold an epitope of about 5-7 amino acids or 3-4 sugar residues

## Antigen Antibody Binding Equilibrium

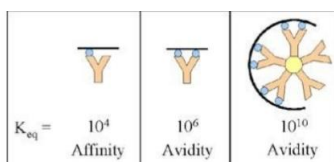


## Affinity

- 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 of the antibody
- Affinity is the equilibrium constant that describes the Ag-Ab reaction as illustrated. Most antibodies have a high affinity for their antigens.

## 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. This is illustrated in the on the next page.



## Specificity

- Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen.
- In general, there is a high degree of specificity in Ag-Ab reactions. Antibodies can distinguish differences in
  - the primary structure of an antigen

- isomeric forms of an antigen
- secondary and tertiary structure of an antigen

### Cross Reactivity

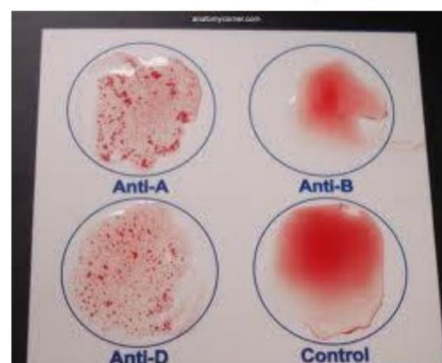
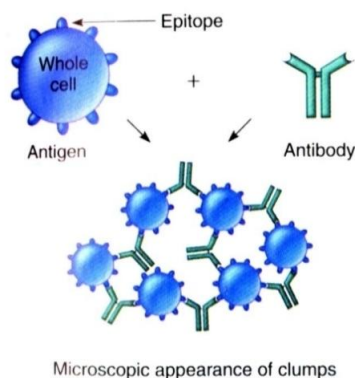
- Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen.
- Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

### Visualizing Antigen-Antibody Reactions

- Agglutination
- Precipitation
- Complement fixation
- Fluorescent antibody tests
- ELISA and RIA
- Western Blot

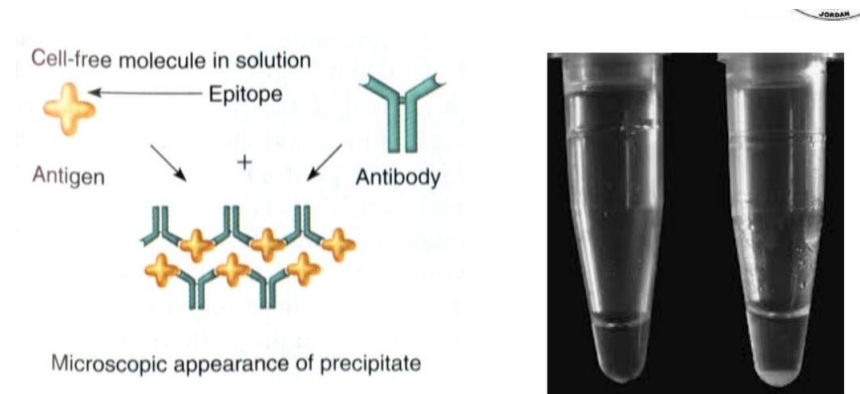
#### 1. Agglutination Testing

- Agglutination: antigens are whole cells such as red blood cells or bacteria with determinant groups on the surface
- Antibodies cross-link the antigens to form visible clumps
- Performed routinely to determine ABO and Rh blood types
- Widal test: tube agglutination test for diagnosing salmonella and undulant fever
- Latex agglutination tests: tiny latex beads with antigens affixed



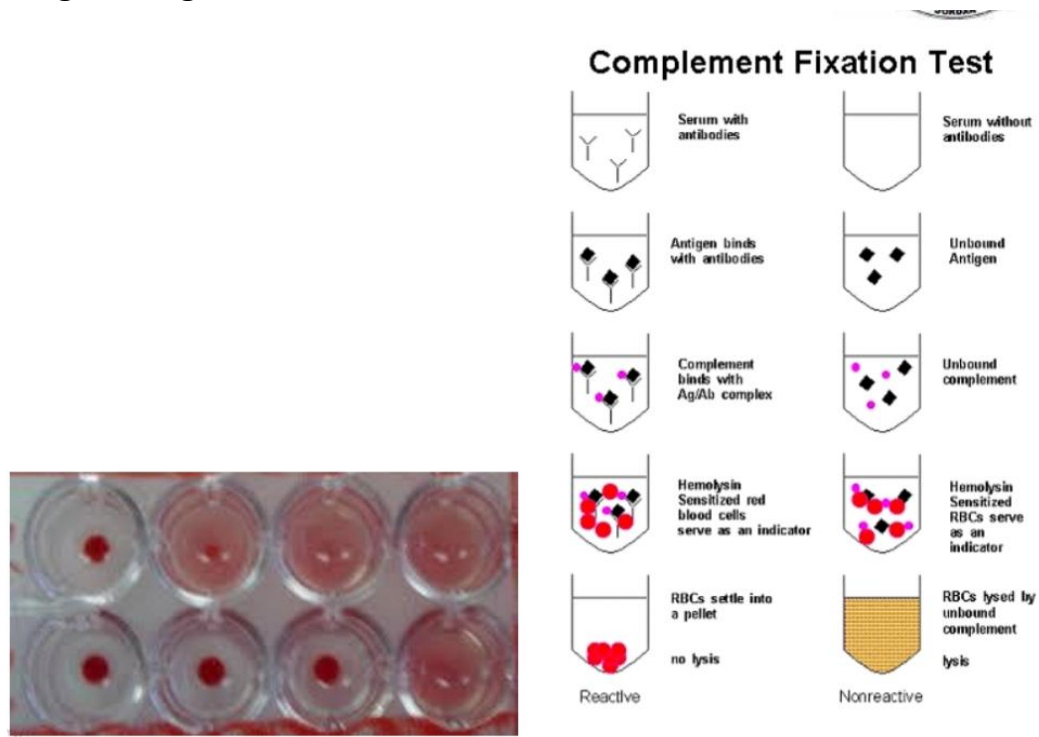
## 2. Precipitation Tests

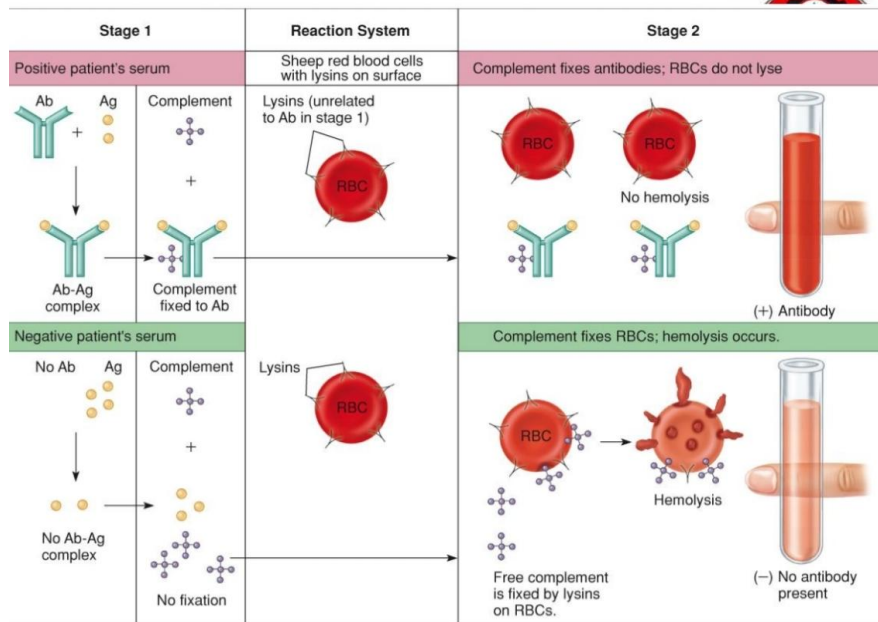
- Precipitation is the interaction of a soluble Ag with a soluble Ab to form an insoluble complex.
- The complex formed is an aggregate of Ag and Ab
- Reaction is observable as a cloudy or opaque zone at the point of contact
- Example: VDRL (Venereal Disease Research Lab) test streptococcal group antigens testing



## 3. Complement Fixation

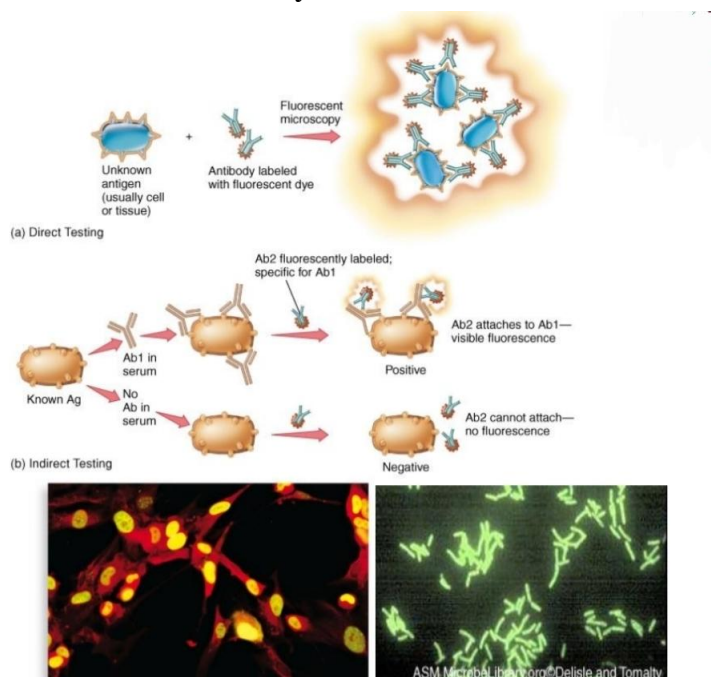
- Lysin or cytotoxin: an antibody that requires complement to complete the lysis of its antigenic target cell





#### 4. Fluorescent Antibodies and Immunofluorescence Testing

- Direct testing: an unknown test specimen or antigen is fixed to a slide and exposed to a fluorescent antibody solution of known composition
- Indirect testing: the fluorescent antibodies are antibodies made to react with the Fc region of another antibody



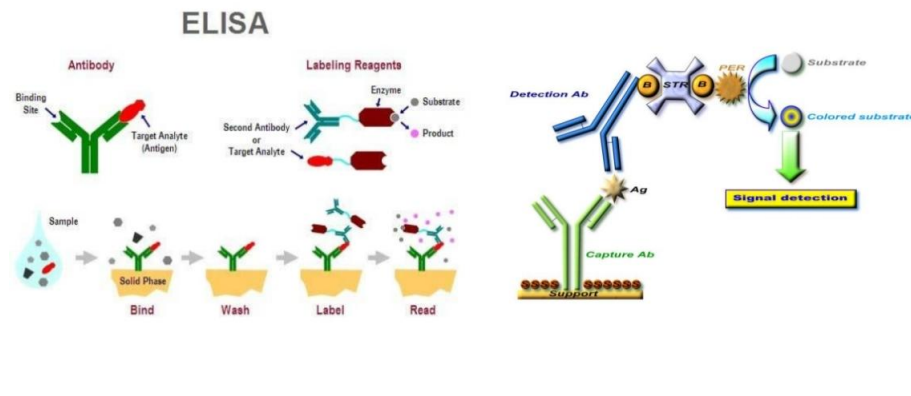
#### 5. Radioimmunoassay (RIA)

##### Enzyme-Linked Immunosorbent Assay (ELISA)

- Antibodies or antigens labeled with a radioactive isotope (RIA) or Enzyme (ELISA) used to pinpoint minute amounts of a corresponding antigen or antibody

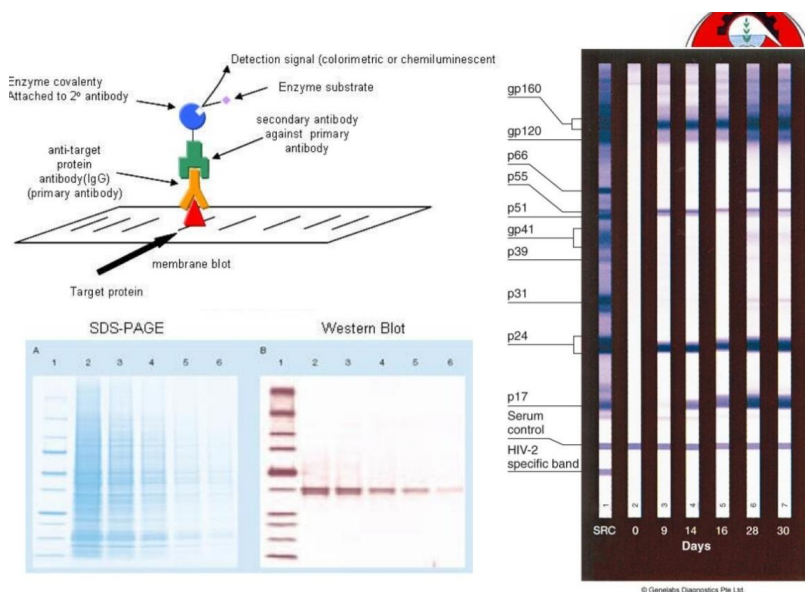


- Compare the amount of radioactivity present in a sample before and after incubation with a known, labeled antigen or antibody



## 6. The Western Blot for Detecting Proteins

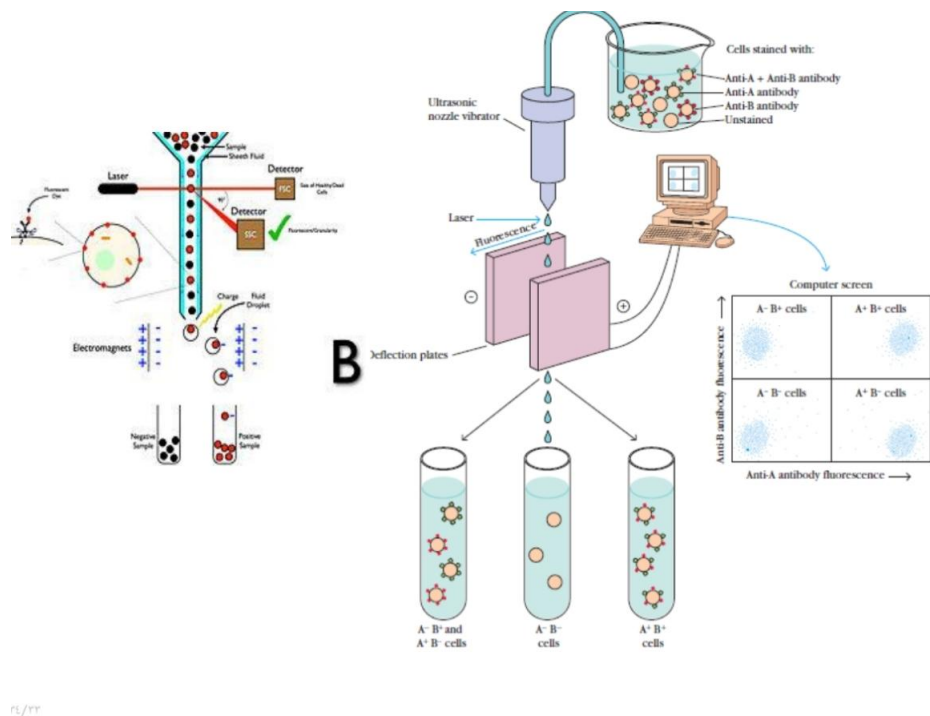
- Test material is electrophoresed in a gel to separate out particular bands
- Gel transferred to a special blotter that binds the reactants in place
- Blot developed by incubating it with a solution of antigen or antibody labeled with radioactive, fluorescent, or luminescent labels



## 7. Flow Cytometry

- The flow cytometer was designed to automate the analysis and separation of cells stained with fluorescent antibody
- The flow cytometer uses a laser beam and light detector to count single intact cells in suspension
- Every time a cell passes the laser beam, light is deflected from the detector, and this interruption of the laser signal is recorded
- Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam

- It has large number of medical application for example in classification and treatment of leukemias



- **Leukocyte adhesion deficiency (LAD)** is a rare genetic disorder that affects the immune system. It is characterized by a defect in the ability of white blood cells (leukocytes) to properly adhere to the blood vessel walls and migrate to sites of infection or inflammation. This condition leads to a compromised immune response, making affected individuals more susceptible to recurrent and severe bacterial and fungal infections.
- **Leukocyte adhesion deficiency (LAD)** is categorized into three main types: LAD-I, LAD-II, and LAD-III.

Each type is associated with specific genetic mutations that result in distinct clinical manifestations. Here's an overview of each type:

#### **-LAD type 1:**

**Characteristics:** mutation in CD 18 gene ( this gene encoding B chain of B2 integrins )

**Clinical features:** umbilical cord detachment , no pus formation, recurrent infection by staph and pseudomonas

#### **-LAD type 2:**

**Characteristics:** Absence of sialyl-Lewis X or E selectin

**Clinical presentation:** pneumonia, TB, recurrent skin infection ,bronchiectasis



**-LAD type 3:**

**Defect in B chain of integrin 1&2&3**

**Clinical presentation: osteoporosis ,bleeding complications,  
Omphalitis (infection of the umbilical cord)**

**TREATMENT**

- **stem cell transplant**
- **INF- $\gamma$**
- **ustekinumab for P40 subunit ( stimulation of IL12 and IL23)**

**Done by: Dr.mohammed farhoud**

**A patient presents with an increased number of lymphocytes in his peripheral blood. Which of the following is an appropriate technique to find out which type of lymphocytes these are?**

- A. Enzyme-linked immunosorbent assay (ELISA).**
- B. Radioimmunoassay (RIA).**
- C. Western blotting.**
- D. Flow cytometric analysis.**
- E. Immunofluorescent staining.**

**A kidney biopsy specimen taken from a patient with acute glomerulonephritis and stained with fluorescein-conjugated anti-human IgG antibody would probably show:**

- A. Patchy, irregular fluorescence of the glomerular basement membrane.**
- B. Uniform fluorescence of the glomerular basement membrane.**
- C. Fluorescent B cells.**
- D. Fluorescent Macrophages.**
- E. No fluorescence.**

**In agglutination reactions, the antigen is ..... in precipitation reactions, the antigen is .....**

- A. whole cell/soluble molecule**
- B. Soluble molecule/whole cell**
- C. Bacterium/virus**
- D. Protein/carbohydrates**
- E. Protein/Antibody**

**Ans:D/A/A**