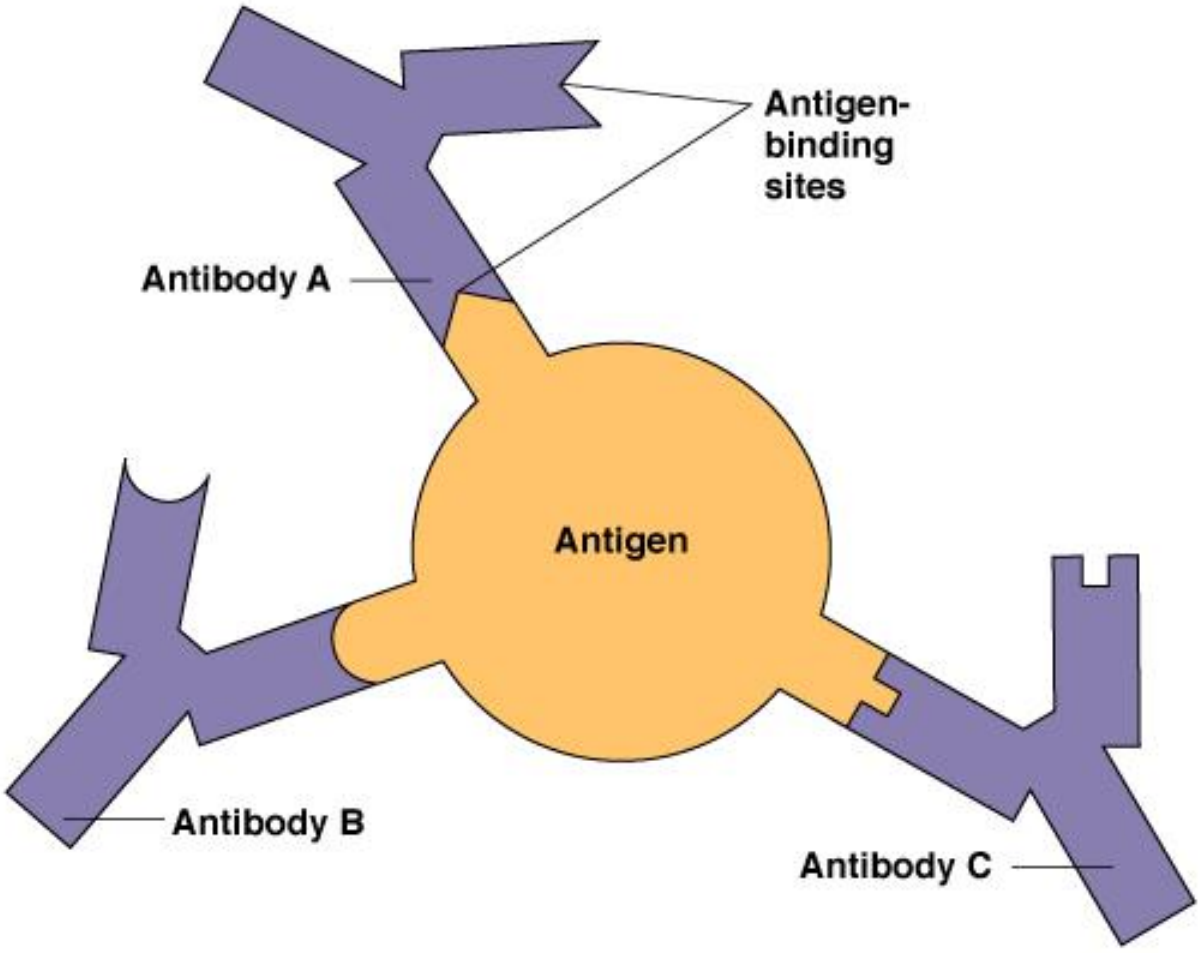
Antigen Antibody Reaction

Objectives

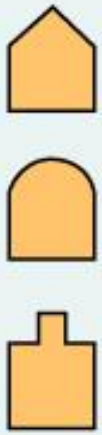
- Discussion of general principles of antigen-antibody interactions
- Definition and importance of affinity, avidity, and cross reactivity
- Laboratory methods used for visualizing antigen-Antibody Reactions

Definitions

- Antigen: Any chemical that creates immune response, most are proteins or large polysaccharides
  - Microbes: Capsules, cell walls, toxins, viral capsids,
  - Non microbes: Pollen, egg white
- Antibodies: Immunoglobulines that recognize and bind to a particular antigen with high specificity and 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



Epitopes
(antigenic
determinants)



Antibody-Antigen Interaction

- The interaction of the antibody with an antigen causes a change in shape of the antibody. This is known as conformational change or induced fit.

- **The Binding Site (Paratope):** Antibodies have a specific binding site for an antigen called the paratope. This site is located at the tips of the Fab (fragment antigen-binding) regions of the antibody.

- **Lock-and-Key vs. Induced Fit**: The initial concept of antibody-antigen binding was based on the "lock-and-key" model, where the antibody's binding site was pre-formed and perfectly complementary to the antigen, like a key fitting into a lock. However, it's now understood that antibodies often undergo conformational changes upon antigen binding.

- **Induced Fit**: In the induced fit model, the antibody's binding site is not perfectly complementary to the antigen before binding. Instead, when the antibody encounters its antigen, the interaction induces a change in the shape of the antibody, allowing the binding site to "mold" itself around the antigen.

Antibody-Antigen Interaction

- **Consequences** of Conformational Change: This shape change has several important consequences:

- **Increased Affinity**: The conformational change often results in a tighter and more stable binding between the antibody and the antigen, increasing the affinity of the interaction.

- **Allosteric Effects**: The shape change can propagate through the antibody molecule, affecting other parts of the antibody, such as the Fc region. This can influence the ability of the antibody to interact with other immune components, like complement proteins or Fc receptors on immune cells.

- **Exposure of Hidden Sites**: As mentioned earlier, the conformational change can expose previously hidden sites on the antibody (e.g., the C1q binding site on IgG, which is necessary for complement activation).

Antibody-Antigen Interaction

- May cause the exposure of another site which then is responsible for the various reactions elicited by the antibody to destroy the foreign substance such as complement fixation.

- The interaction of antibodies and antigens may produce a network-type complex known as an immune complex. More specifically, the process of forming these networks is called immune complex formation or cross-linking, and when it becomes visible it's often referred to as agglutination or precipitation, depending on the nature of the antigen.

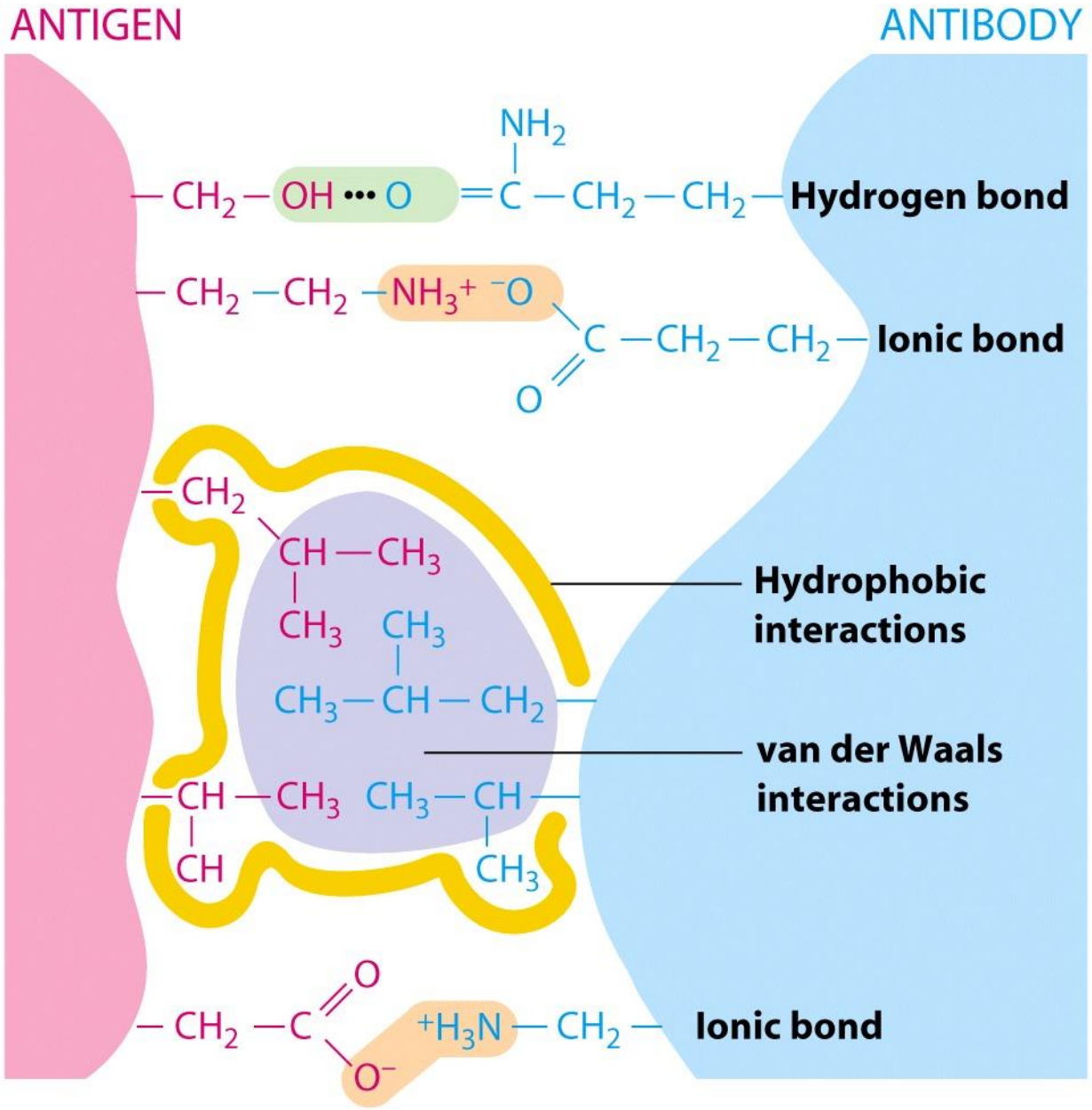
Nature of Antigen Antibody Reaction

- **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.

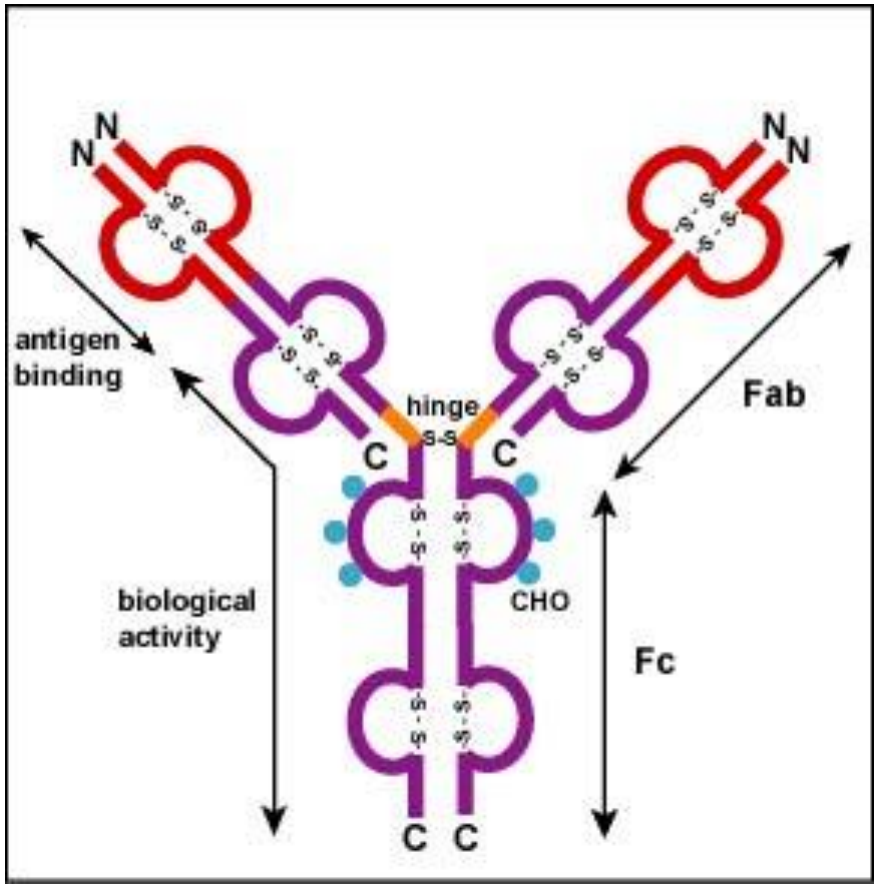
- **Reversibility**: Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible

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 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. Most antibodies have a high affinity for their antigens.
- A higher affinity means a stronger interaction.

Antibody Avidity and Valance

- **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.
- **Valence**: This refers to the **number** of binding sites.

➢ **Antibody Valence**: IgG, for example, is divalent (valence of 2) because it has two Fab regions, each capable of binding an antigen. IgM is pentameric (valence of 10, though sterically hindered), meaning it has five antibody monomers joined together, providing ten antigen-binding sites.

➢ **Antigen Valence**: An antigen's valence refers to the number of repeating epitopes it presents. A large, complex antigen (e.g., a bacterial cell with numerous identical surface proteins) will have high valence.

- **Avidity**: This is the overall strength of the interaction between an antibody and an antigen. It takes into account BOTH the affinity of the individual binding sites AND the number of binding sites involved (valence).
- **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.

Antibody 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.

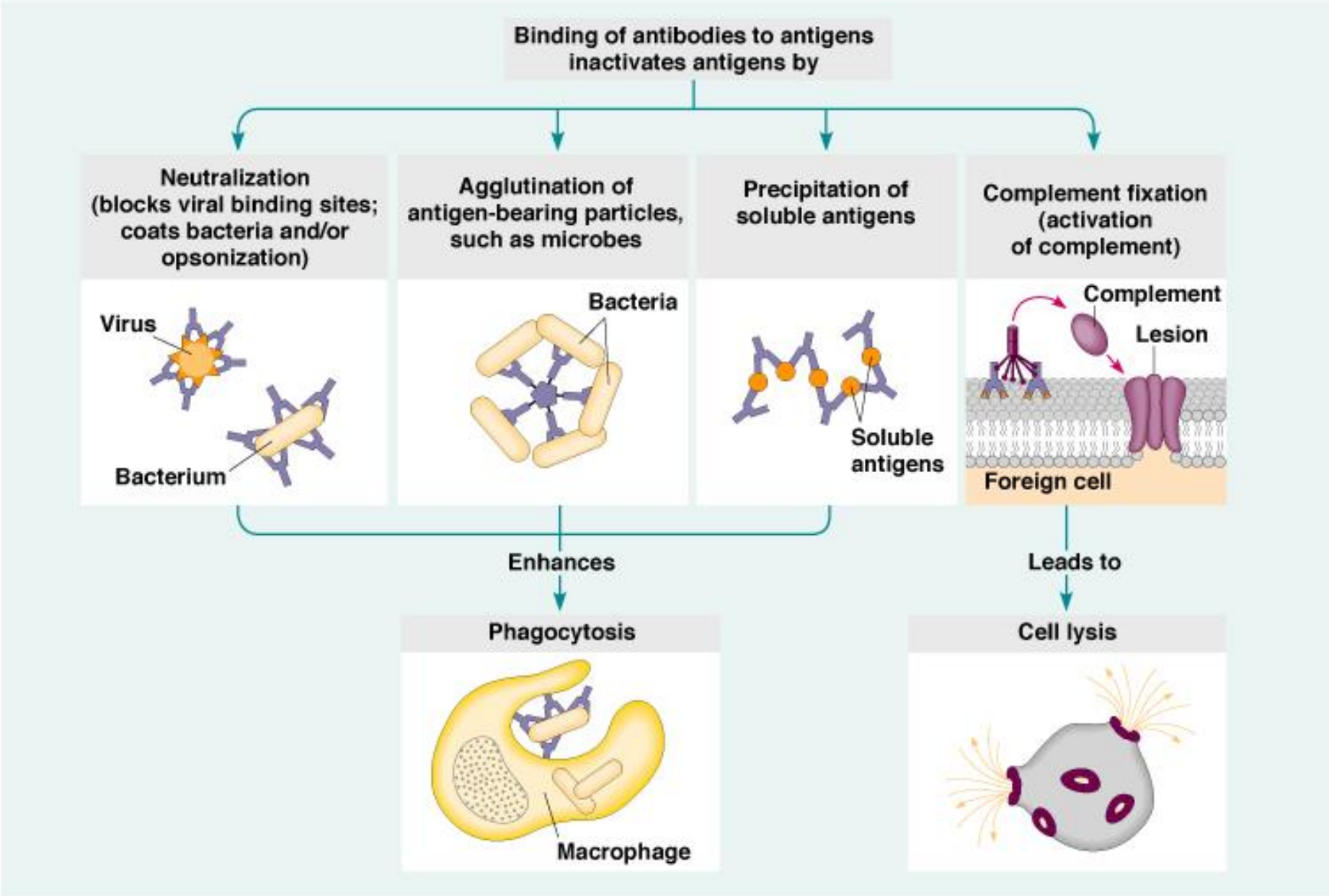
- In general, there is a high degree of specificity in Ag-Ab reactions. Antibodies can distinguish differences in
  - The primary structure of an antigen
  - Isomeric forms of an antigen
  - Secondary and tertiary structure of an antigen

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 (multi-specificity).

Consequences of Antibody Binding

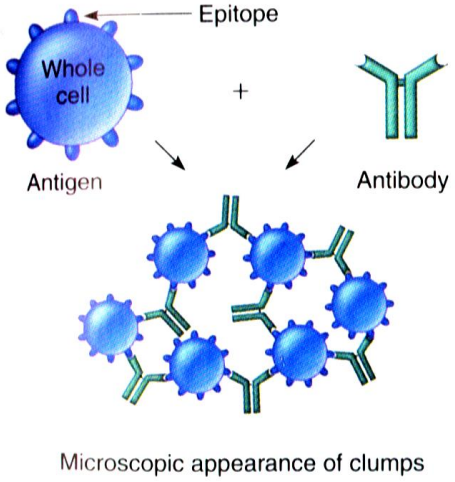


Visualizing Antigen-Antibody Reactions

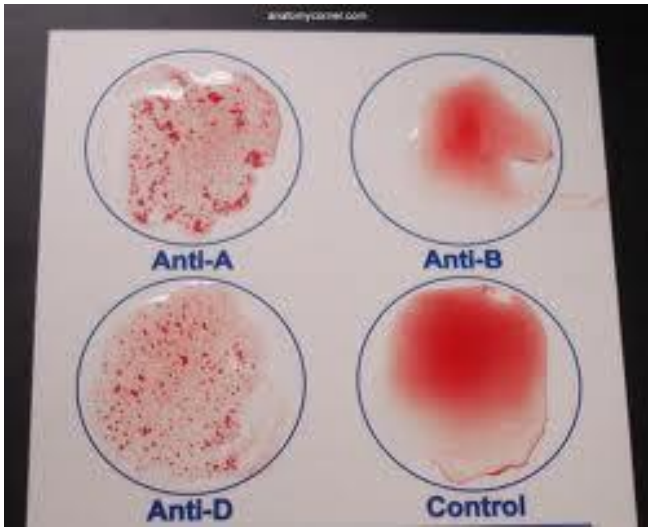
- Agglutination
- Precipitation
- Complement fixation
- Fluorescent antibody tests
- ELISA and RIA
- Western Blot

1. Agglutination Testing

- Agglutination means the clumping or aggregation of particle.
- 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 are used for the rapid detection and identification of various antigens and antibodies in biological samples. It is tiny latex beads with antigens affixed.

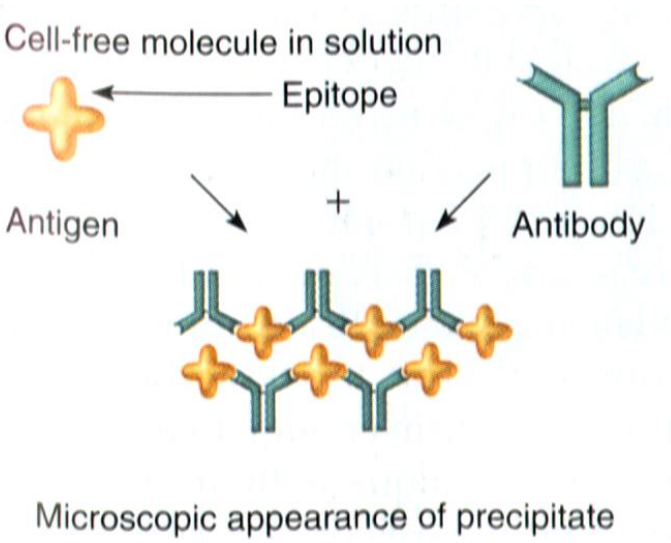


Microscopic appearance of clumps



2. Precipitation Tests

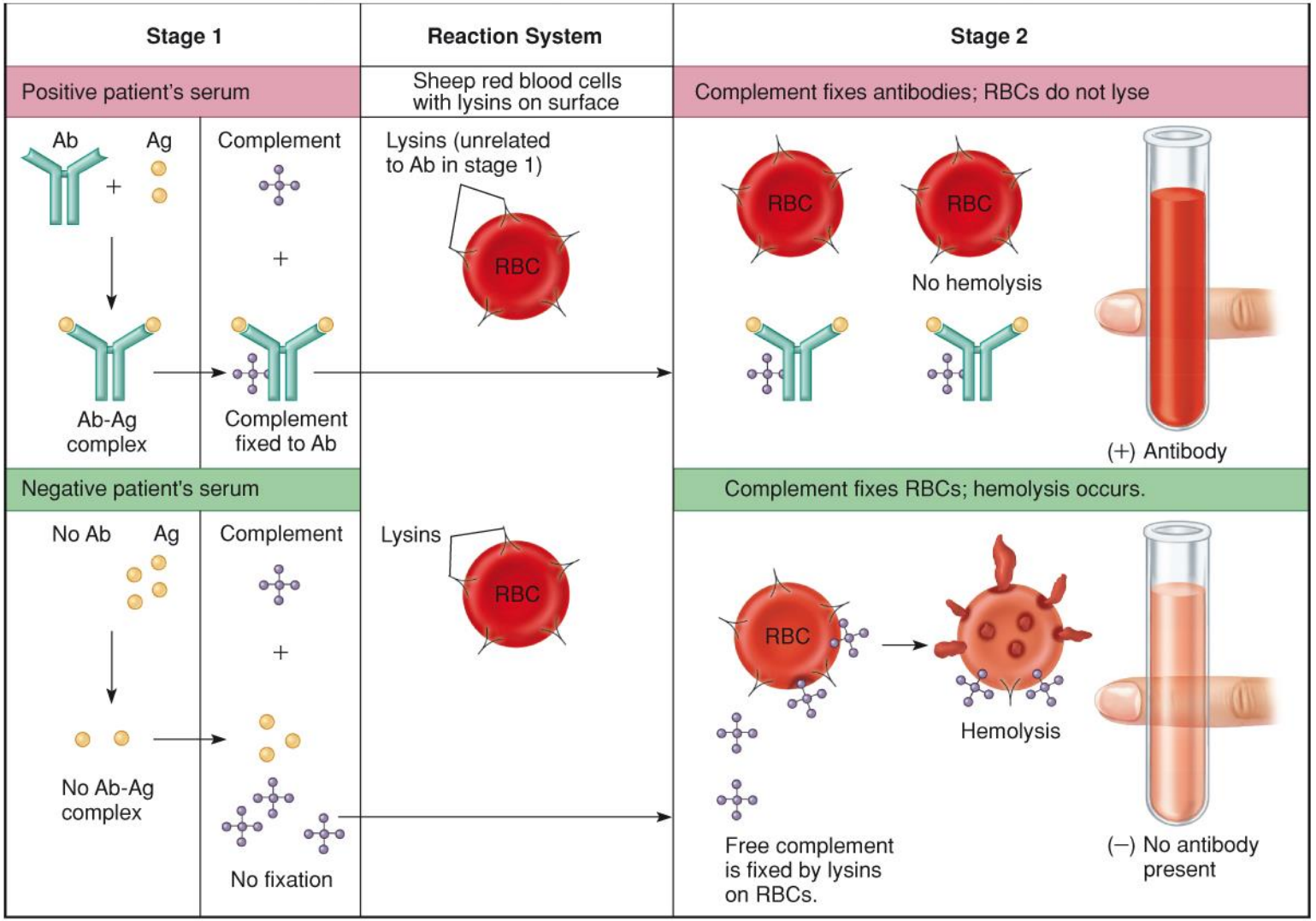
- Precipitation is the interaction of a soluble Ag with a soluble Ab to for an **insoluble complex**.
- The complex formed is an aggregate of Ag and Ab
- Reaction is observable as a cloudy or opaque zone at the point of contact
- Example: VDRL (Veneral Disease Research Lab) test.



Microscopic appearance of precipitate

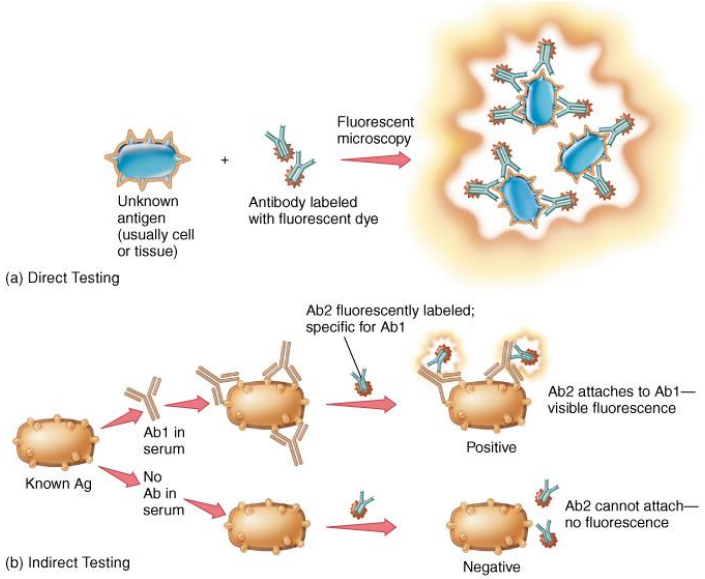
3. Complement Fixation

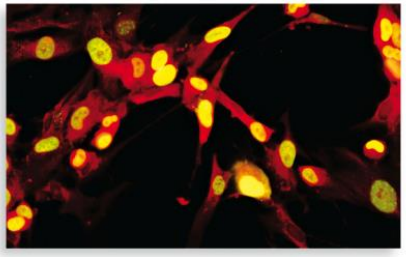
- Lysin or cytolysin: an antibody that requires complement to complete the lysis of its antigenic target cell.

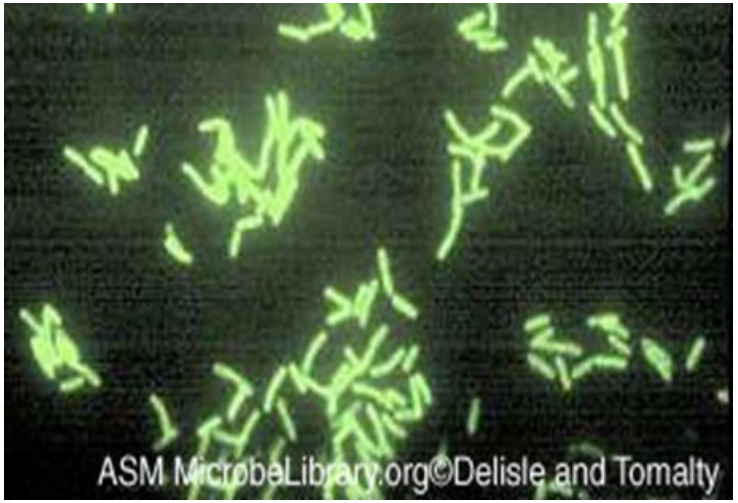


4. Fluorescent Antibodies and Immunofluorescence Testing

- **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

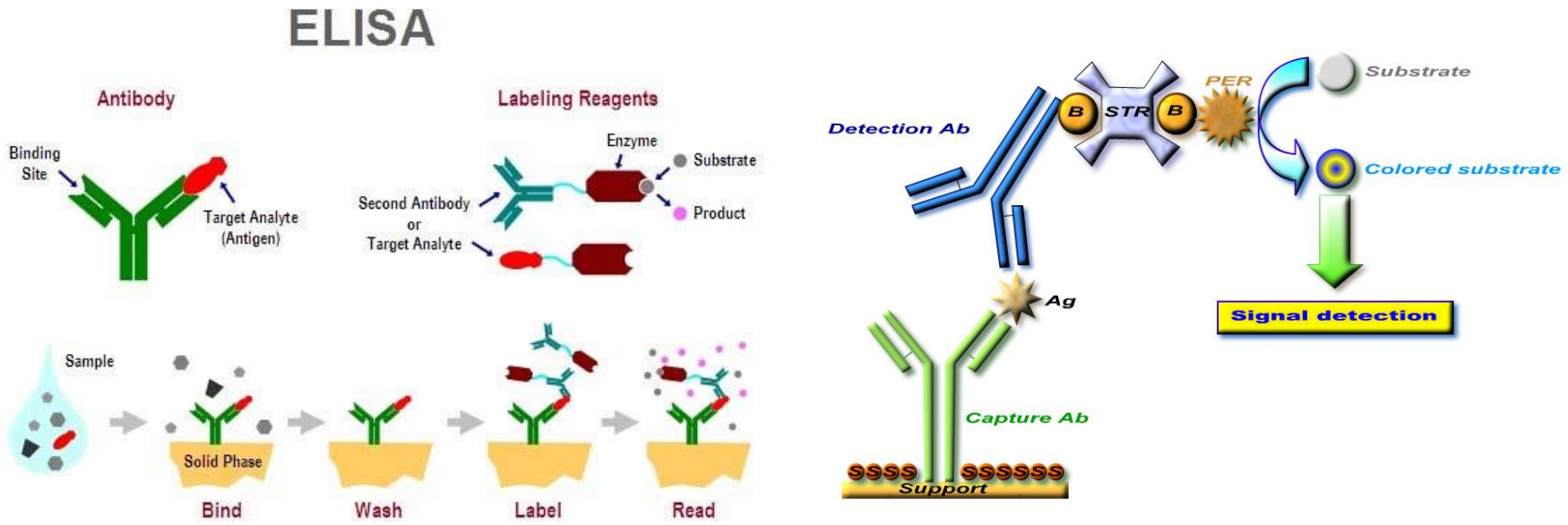






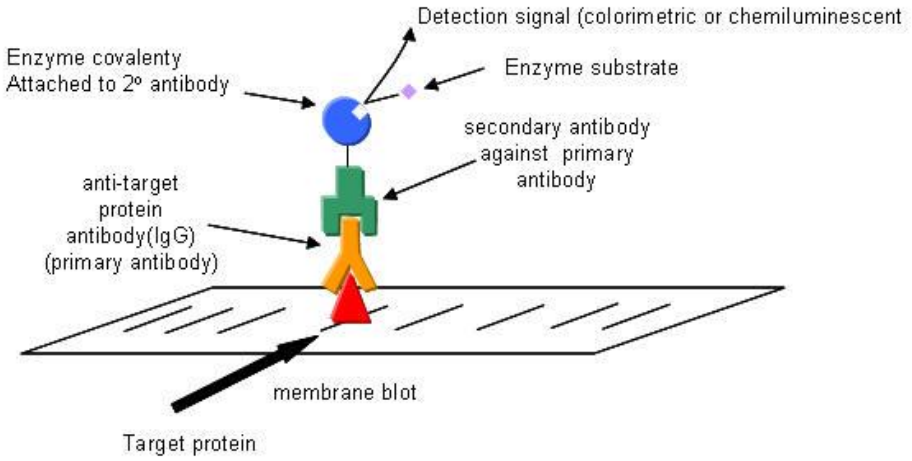
5. Radioimmunoassay (RIA)
Enzyme-Linked Immunosorbent Assay (ELISA)

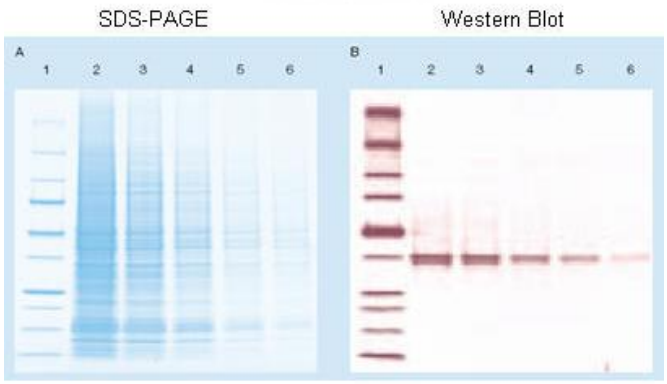
- Antibodies or antigens labeled with a radioactive isotope (RIA) or Enzyme (ELISA) used to pinpoint minute amounts of a corresponding antigen or antibody
- Compare the amount of radioactivity present in a sample before and after incubation with a known, labeled antigen or antibody.

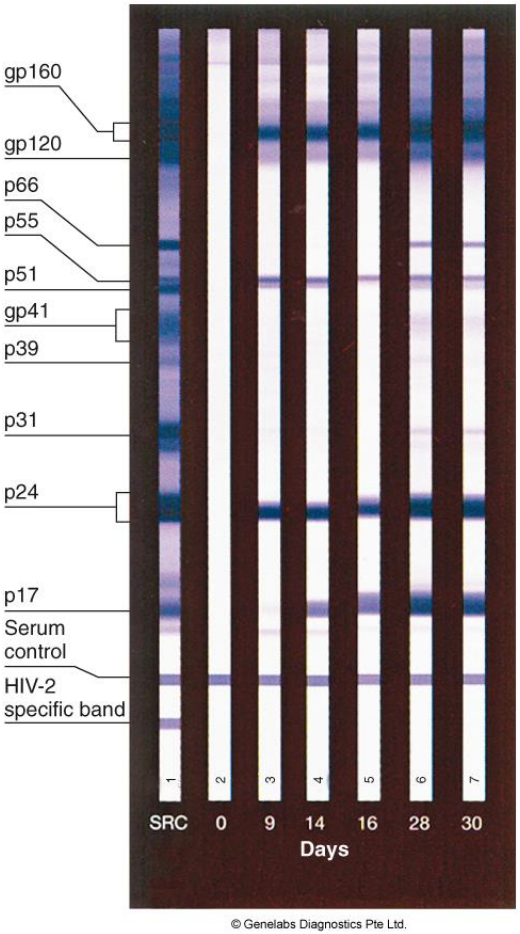


6. The Western Blot for Detecting Proteins

- 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