Antigen Antibody Reaction

Immunology Lecture 6
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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 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

Antigen-binding sites

Antibody A

Antigen

Antibody B

Antibody C

Epitopes (antigenic determinants)

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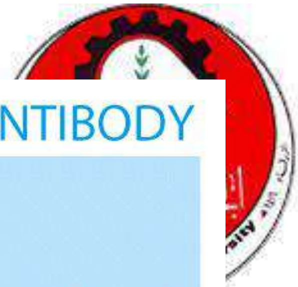
Antibody-Antigen Interaction

- 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

Hydrogen bond

Ionic bond

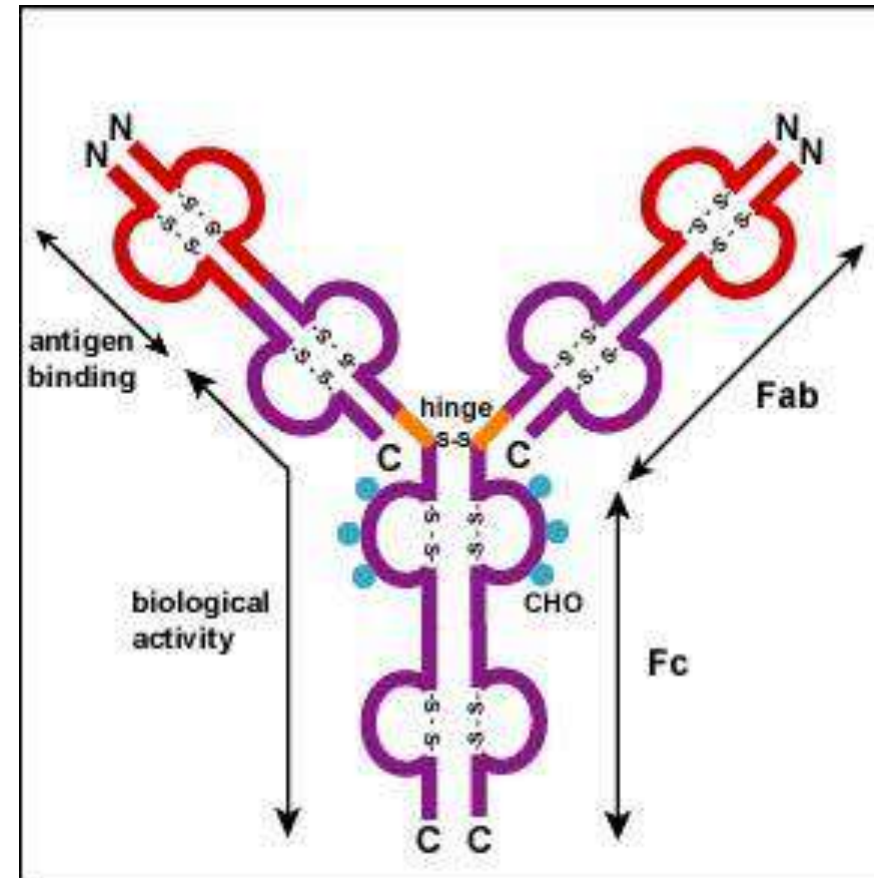
Hydrophobic interactions

van der Waals interactions

Ionic bond

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

Antibody-excess zone

Equivalence zone

Antigen-excess zone

Supernatants: excess Ab, excess Ag

	Antibody-excess zone					Equivalence zone		Antigen-excess zone			
excess Ab	+	+	+	+	±	–	–	–	–	–	–
excess Ag	–	–	–	–	–	–	–	+	+	+	+

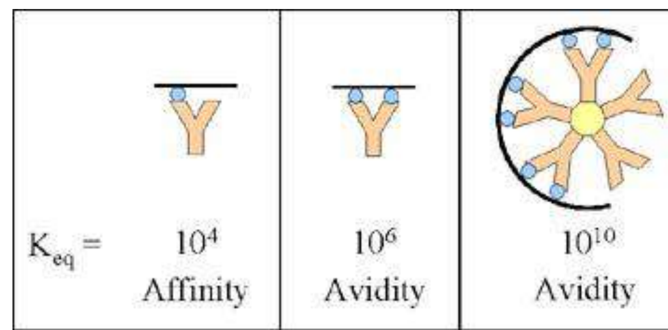
Antibody precipitated

Antigen added

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

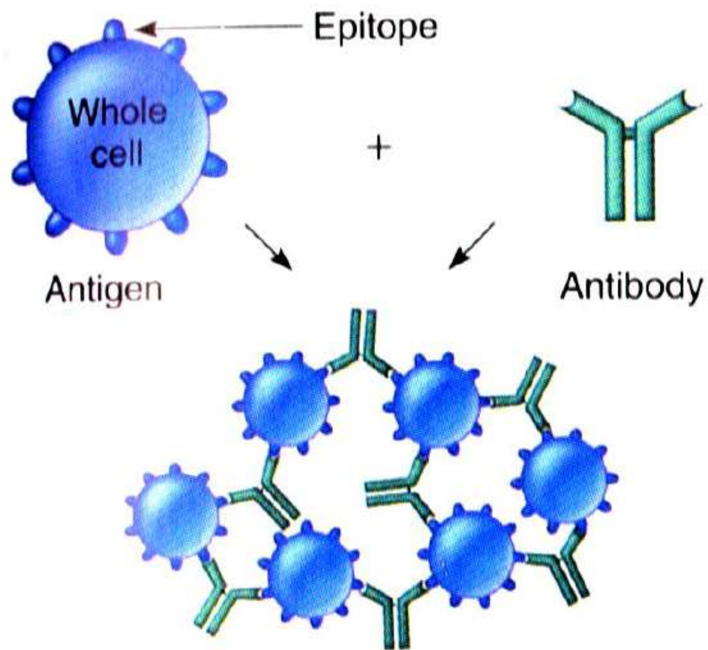
- Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen.
- Cross reactions arise because the cross reacting antigen shares an epitope in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

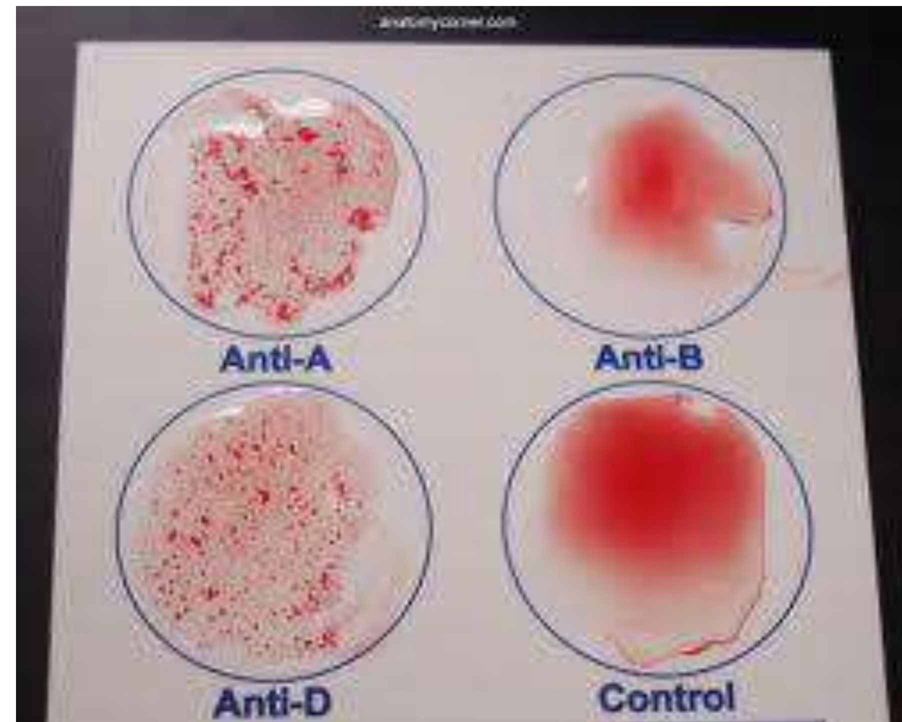
Visualizing Antigen-Antibody Reactions

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

1. Agglutination Testing

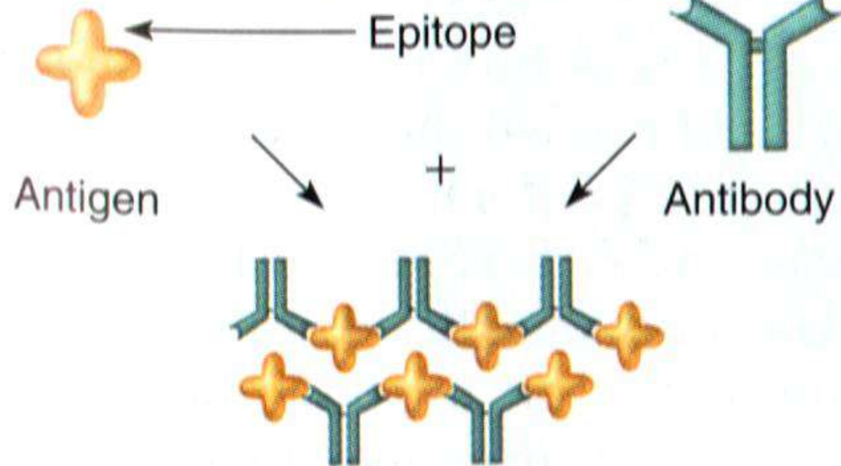
- Agglutination: antigens are whole cells such as red blood cells or bacteria with determinant groups on the surface
- Antibodies cross-link the antigens to form visible clumps
- Performed routinely to determine ABO and Rh blood types
- Widal test: tube agglutination test for diagnosing salmonella and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

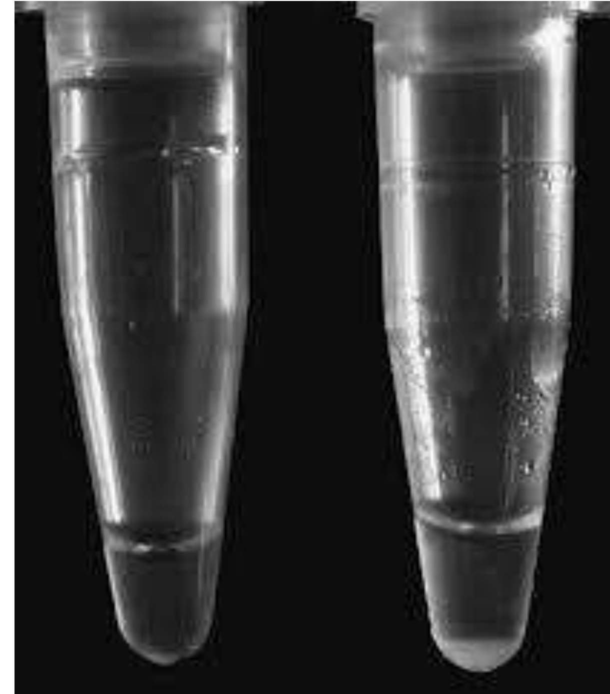

Epitope
Whole cell
+
Antigen
Antibody
Microscopic appearance of clumps


Anti-A
Anti-B
Anti-D
Control

2. Precipitation Tests

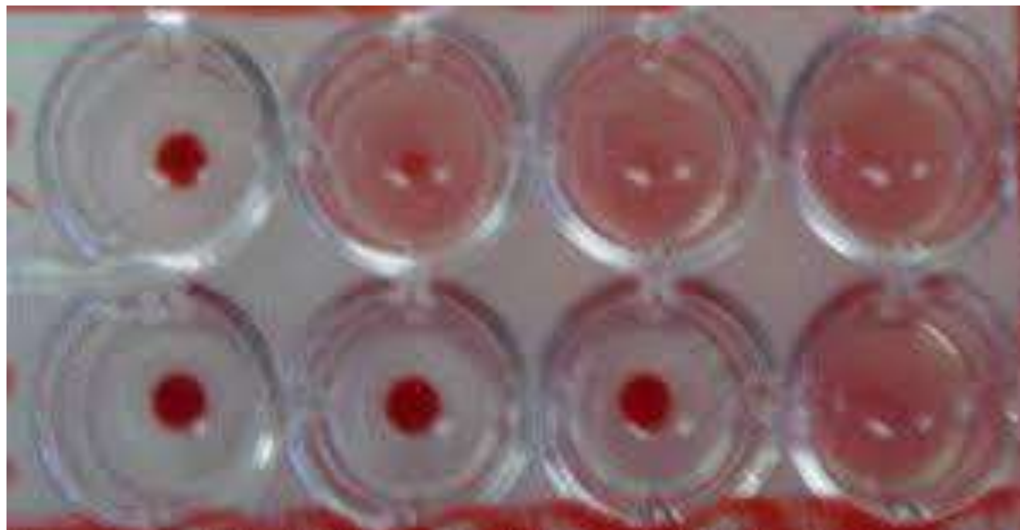
- Precipitation is the interaction of a soluble Ag with a soluble Ab to form an insoluble complex.
- The complex formed is an aggregate of Ag and Ab
- Reaction is observable as a cloudy or opaque zone at the point of contact
- Example: VDRL (Veneral Disease Research Lab) test streptococcal group antigens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


Cell-free molecule in solution
Epitope
Antigen
+
Antibody
Microscopic appearance of precipitate

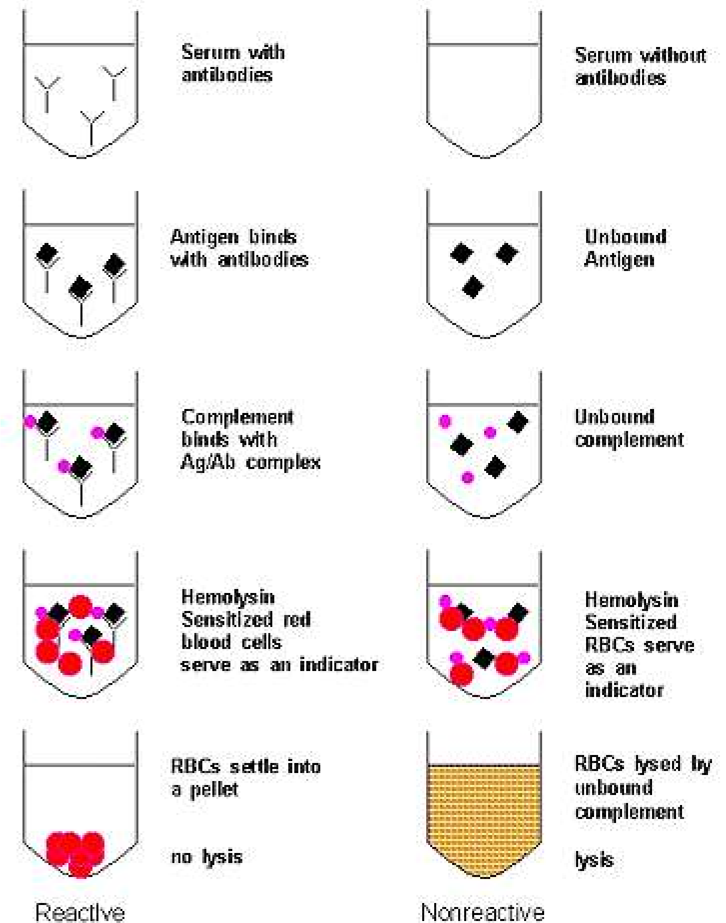


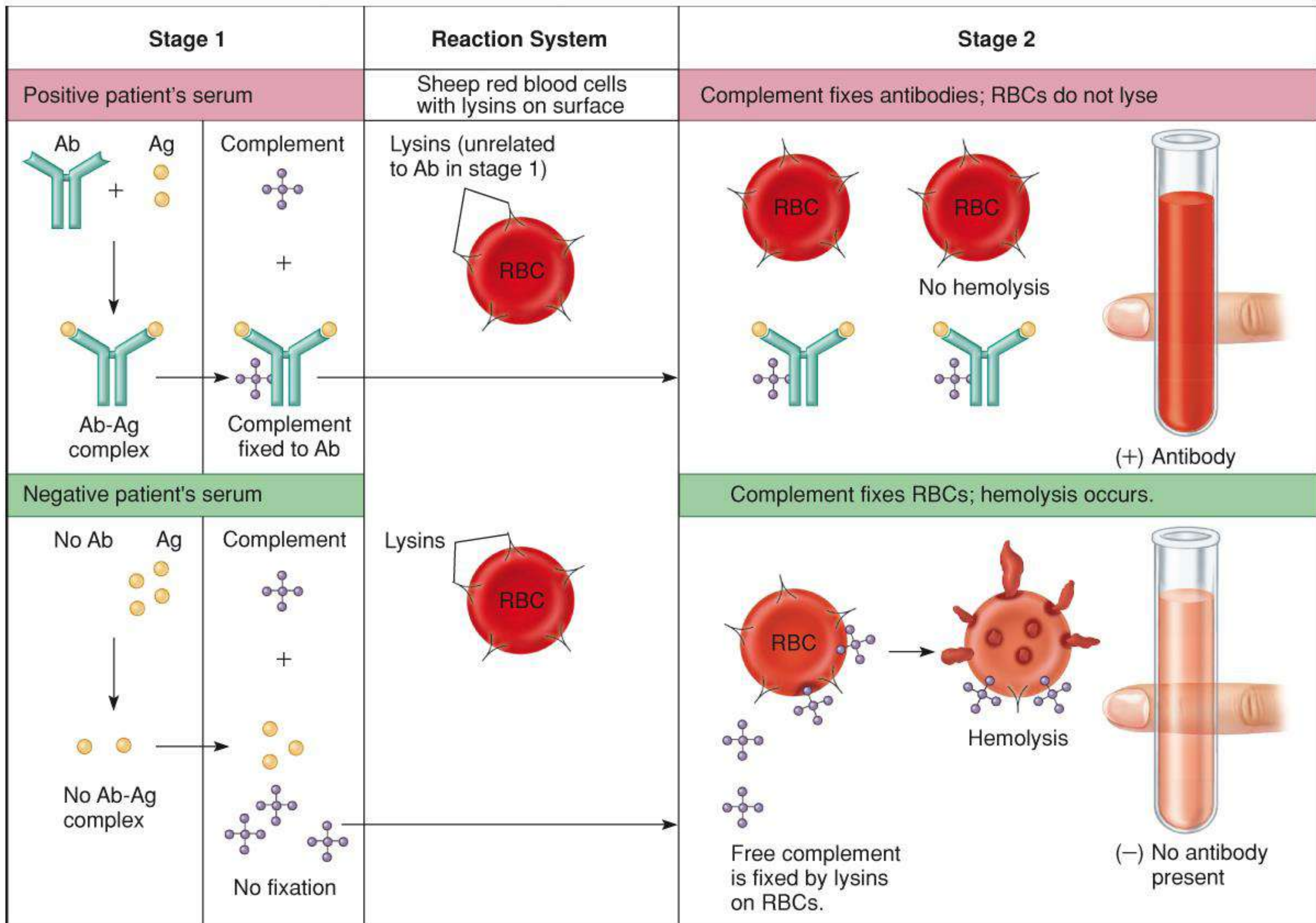
3. Complement Fixation

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



Complement Fixation Test




Stage 1
Reaction System
Stage 2
Positive patient's serum
Sheep red blood cells with lysins on surface
Complement fixes antibodies; RBCs do not lyse
Ab
Ag
Complement
Lysins (unrelated to Ab in stage 1)
RBC
Ab-Ag complex
Complement fixed to Ab
No hemolysis
(+) Antibody
Negative patient's serum
Complement fixes RBCs; hemolysis occurs.
No Ab
Lysins
No Ab-Ag complex
No fixation
Hemolysis
Free complement is fixed by lysins on RBCs.
(–) No antibody present

4. Flurorescent 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

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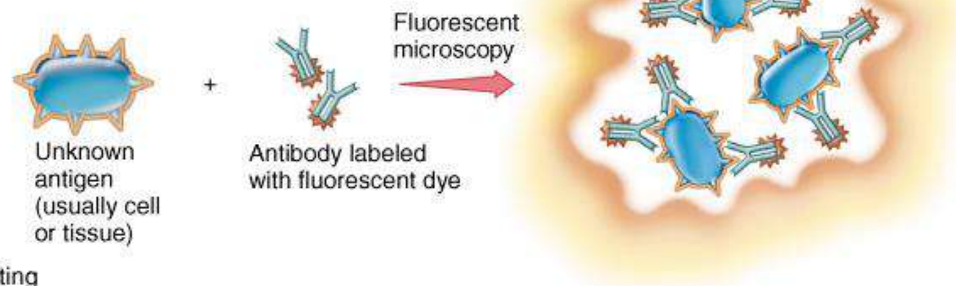
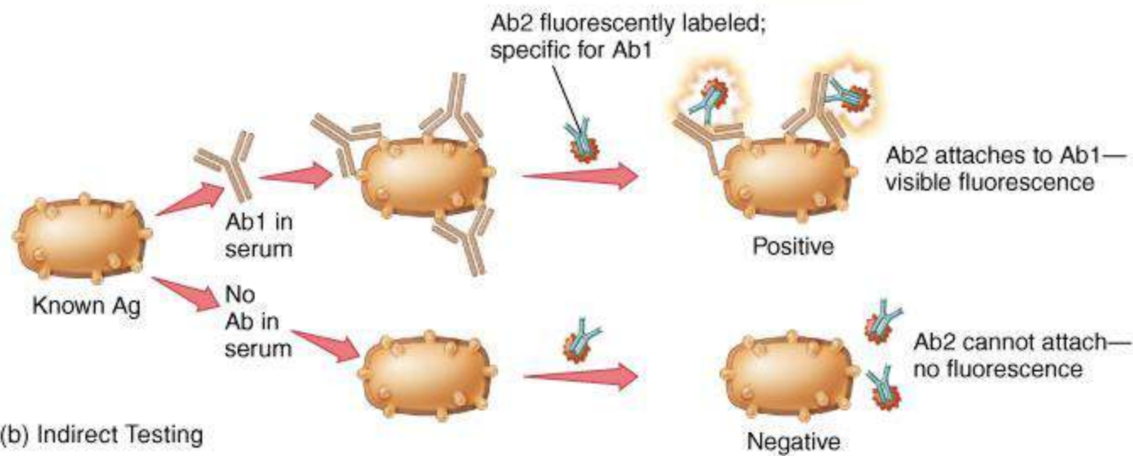
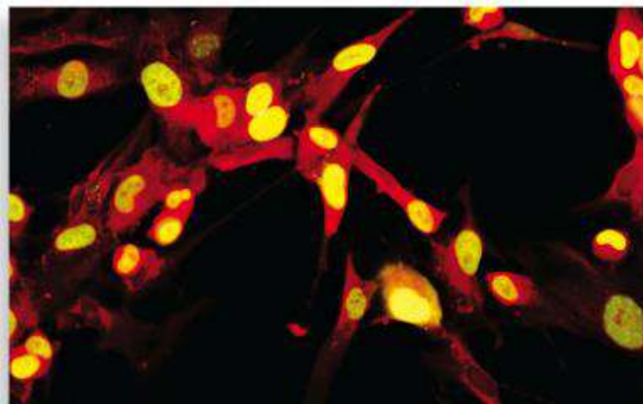
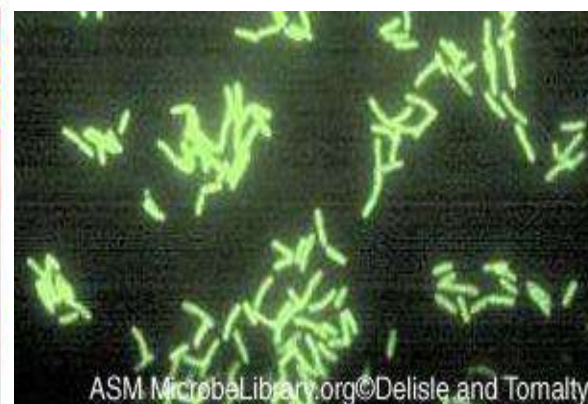


(a) Direct Testing



(b) Indirect Testing

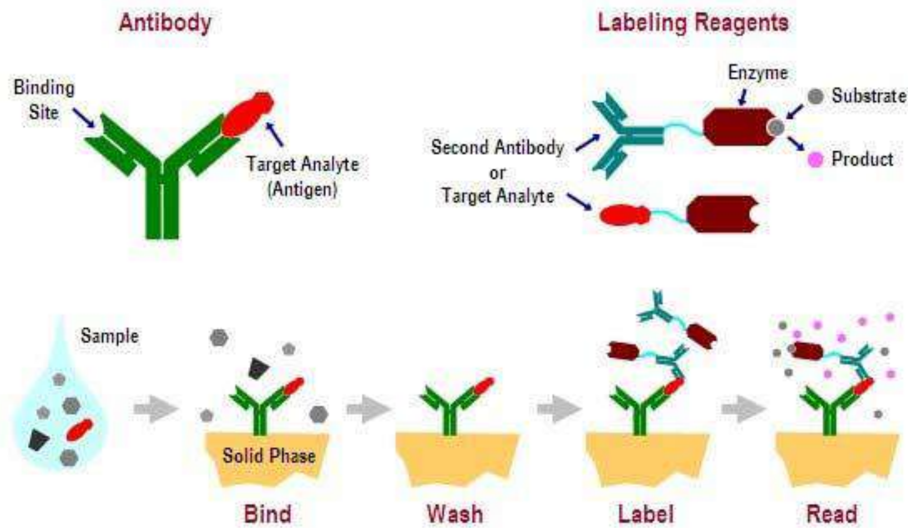


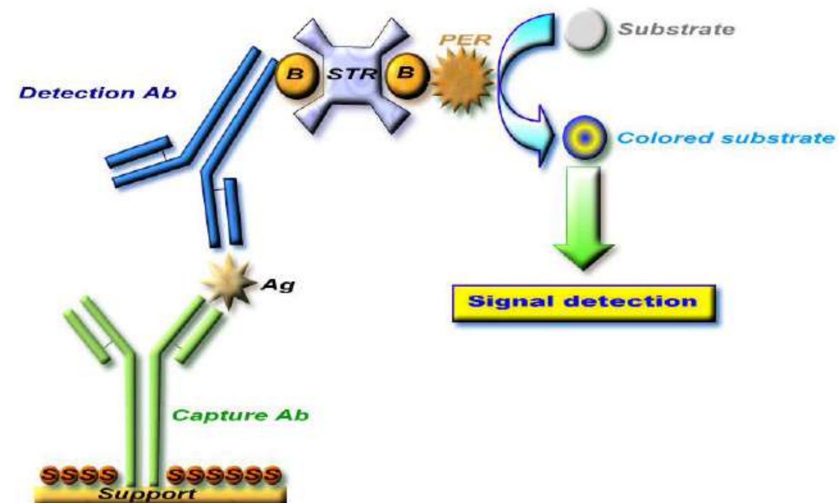


5. Radioimmunoassay (RIA) Enzyme-Linked Immunosorbent Assay (ELISA)

- 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

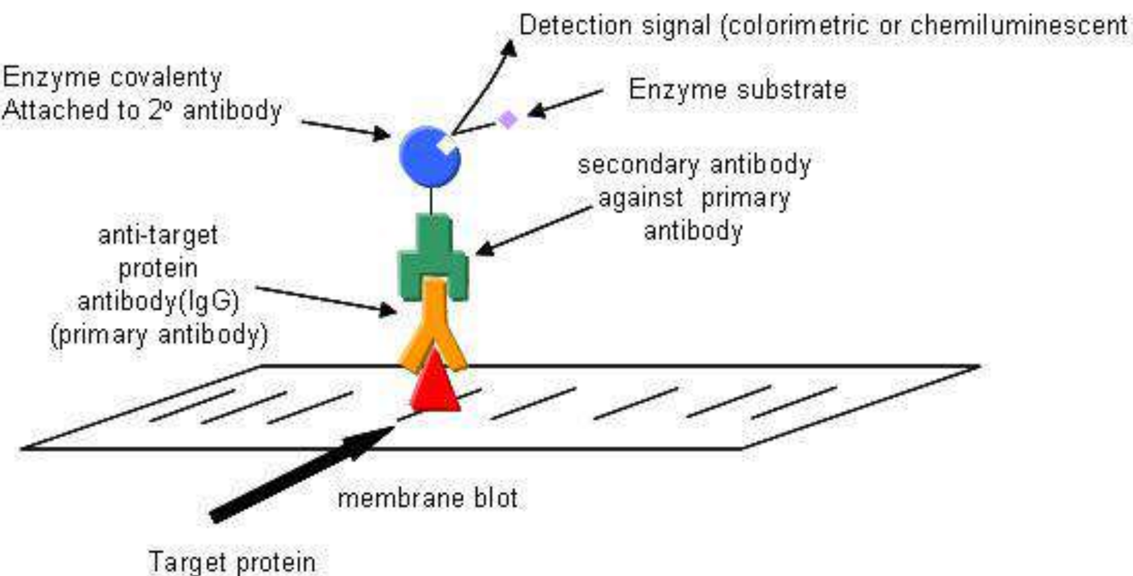
ELISA

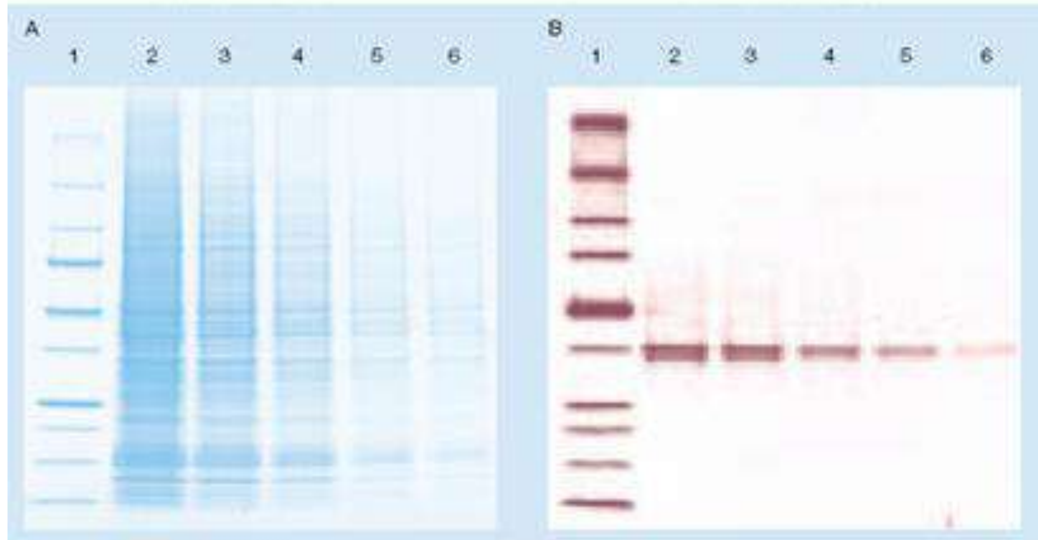


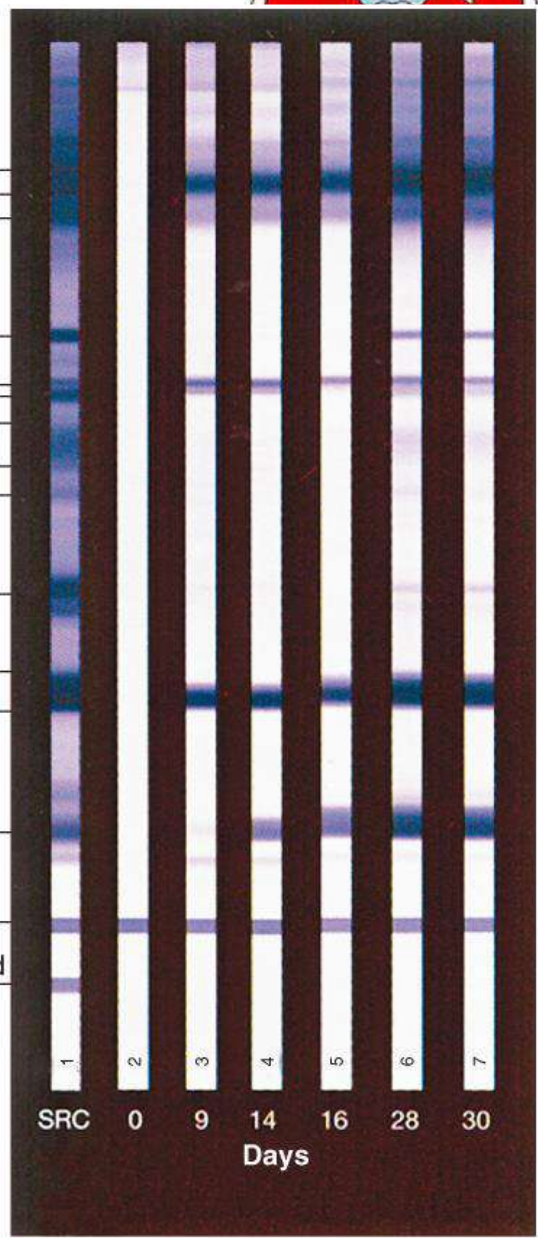


6. The Western Blot for Detecting Proteins

- 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







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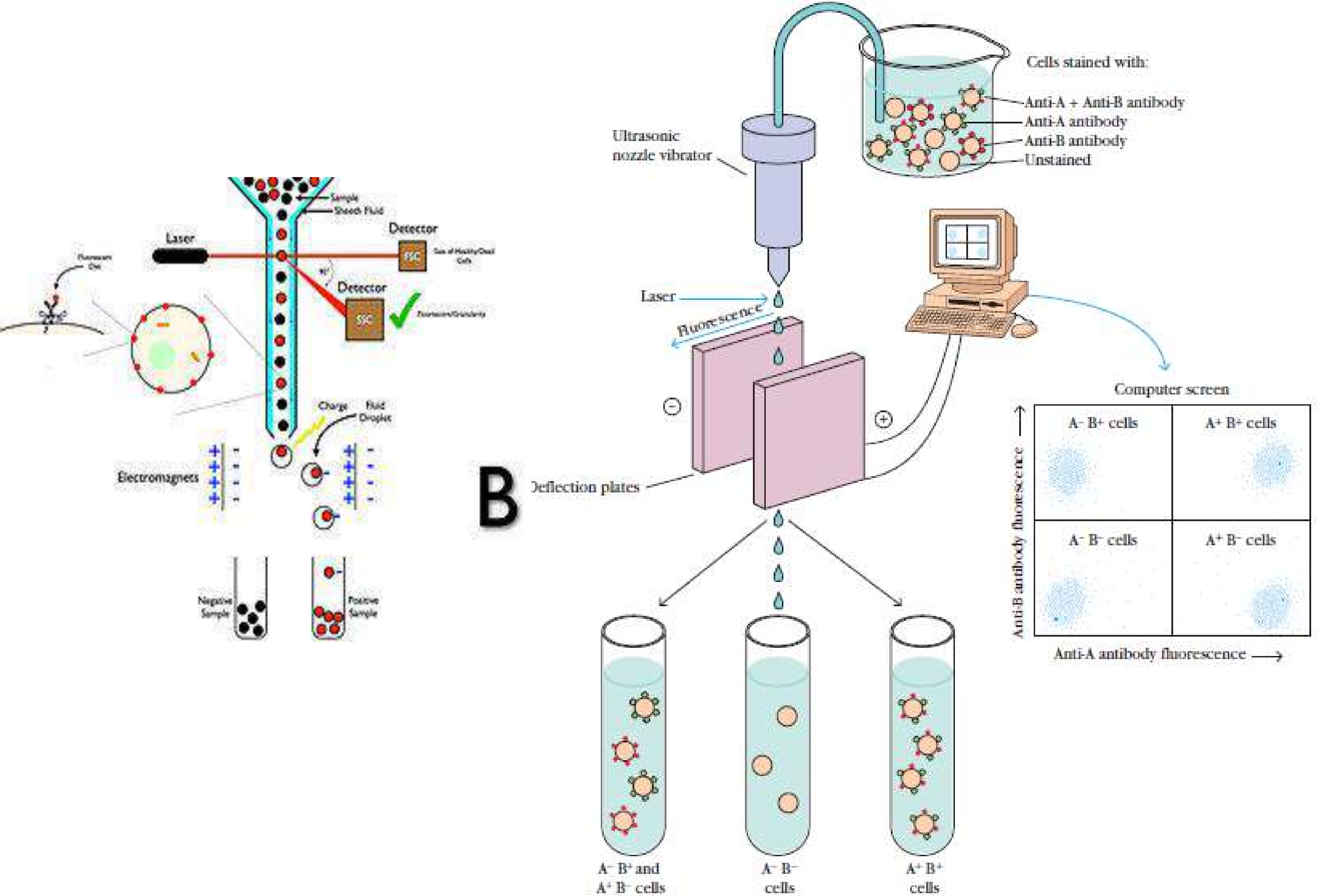
7. Flow Cytometry

- 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


Sample
Sheath Fluid
Laser
Detector
Detector
Charge
Fluid Droplet
Electromagnets
Negative Sample
Positive Sample
B
Ultrasonic nozzle vibrator
Cells stained with:
Anti-A + Anti-B antibody
Anti-A antibody
Anti-B antibody
Unstained
Laser
Fluorescence
Deflection plates
Computer screen
A⁻ B⁺ cells
A⁺ B⁺ cells
A⁻ B⁻ cells
A⁺ B⁻ cells
Anti-B antibody fluorescence
Anti-A antibody fluorescence
A⁻ B⁺ and A⁺ B⁻ cells
A⁻ B⁻ cells
A⁺ B⁺ cells