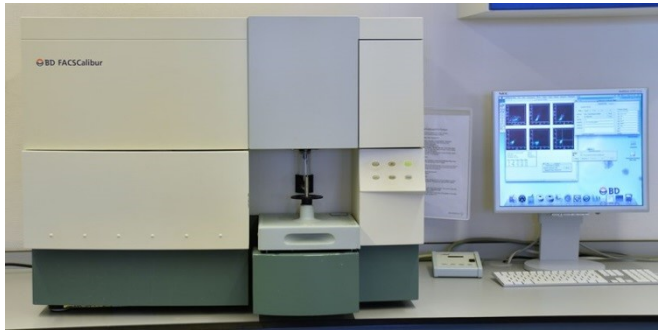
Antigen-Antibody Interactions

Mini Trans-Blot cell.



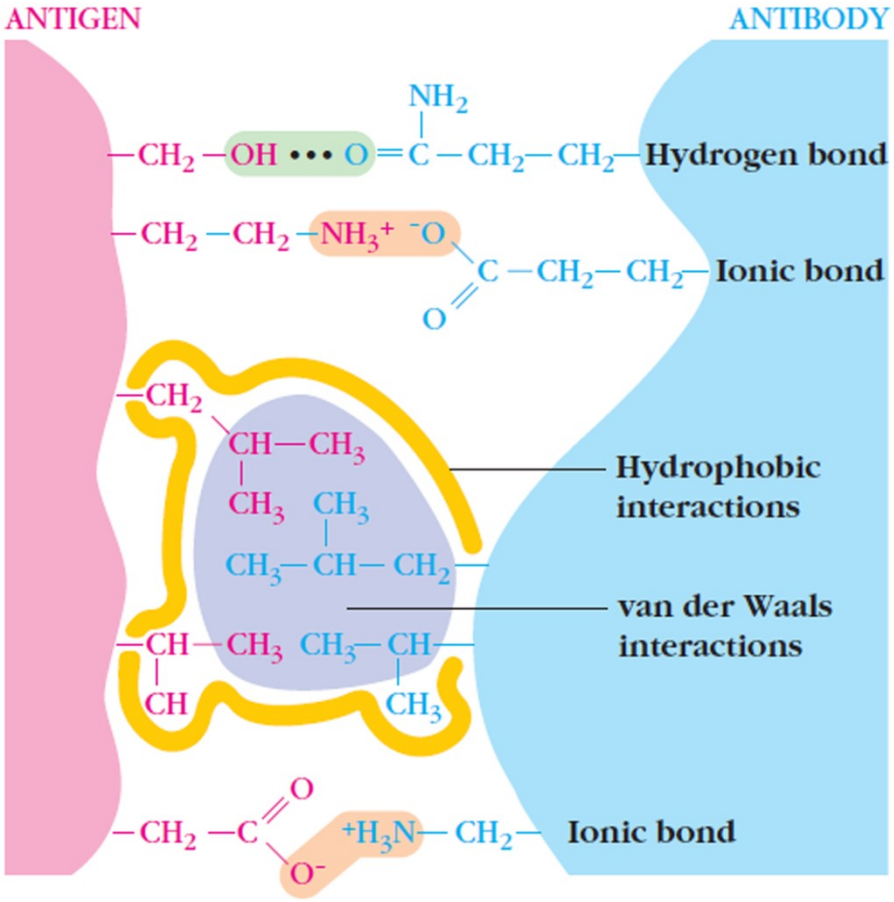
Sudipta Bhowmick MSc PhD
Assistant Professor
Department of Zoology
Dr. Kanailal Bhattacharyya College
Howrah

Antigen-Antibody Interactions

- **The antigen-antibody interaction is a biomolecular association similar to an enzyme-substrate interaction, with an important distinction: it does not lead to an irreversible chemical alteration in either the antibody or the antigen. The association between an antibody and an antigen involves various noncovalent interactions between the antigenic determinant, or epitope, of the antigen and the variable-region (VH/VL) domain of the antibody molecule, particularly the hypervariable regions, or complementarity-determining regions (CDRs).**

- **The exquisite specificity of antigen-antibody interactions has led to the development of a variety of immunologic assays, which can be used to detect the presence of either antibody or antigen. Immunoassays have played vital roles in diagnosing diseases, monitoring the level of the humoral immune response, and identifying molecules of biological or medical interest. These assays differ in their speed and sensitivity; some are strictly qualitative, others are quantitative.**

Antigen-Antibody Interactions



Antibody Affinity Is a Quantitative Measure of Binding Strength

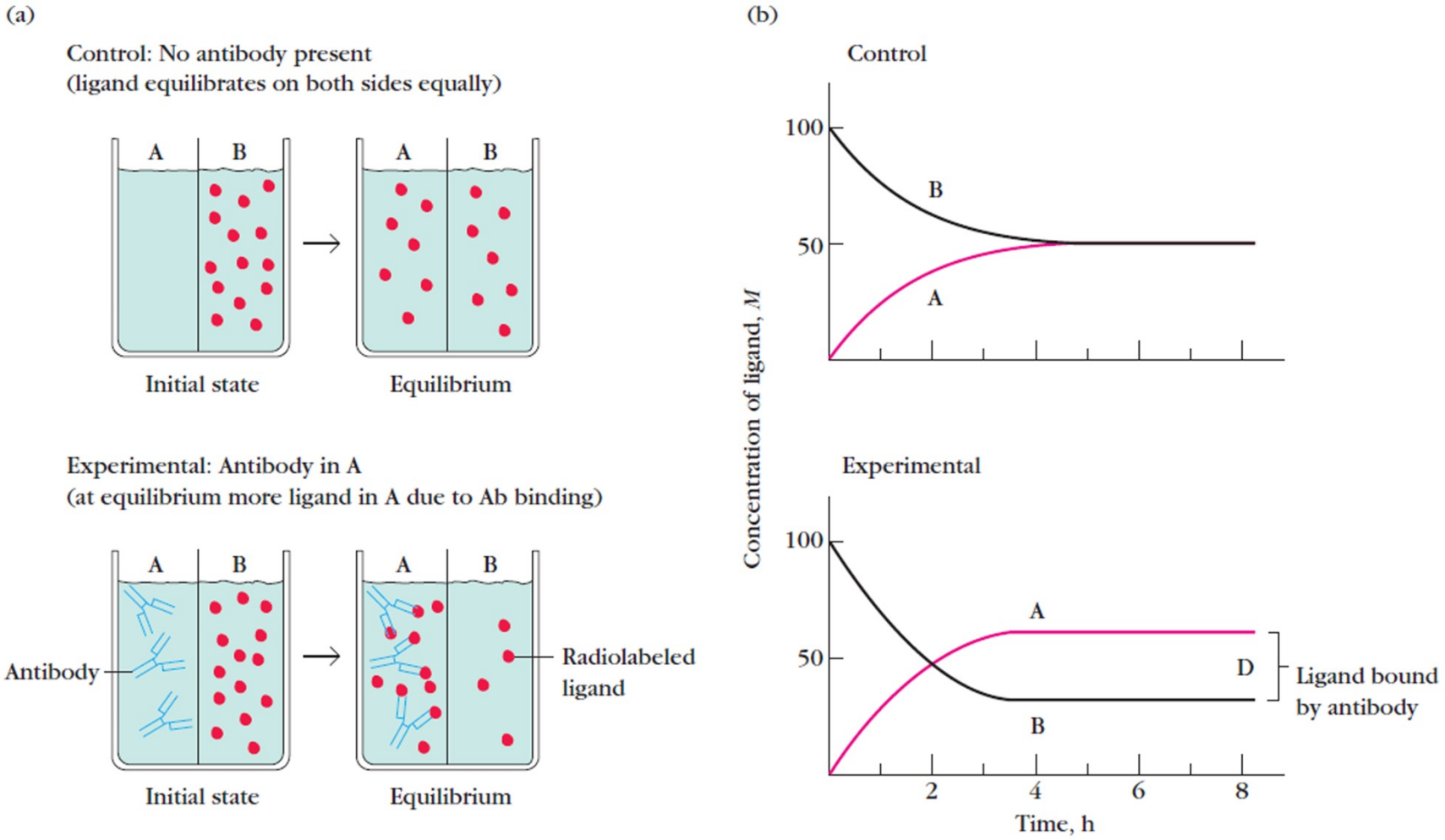
- The combined strength of the noncovalent interactions between a *single* antigen-binding site on an antibody and a *single* epitope is the affinity of the antibody for that epitope. Low-affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer. The association between a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation

$$\mathrm{Ag} + \mathrm{Ab} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ag\text{-}Ab}$$

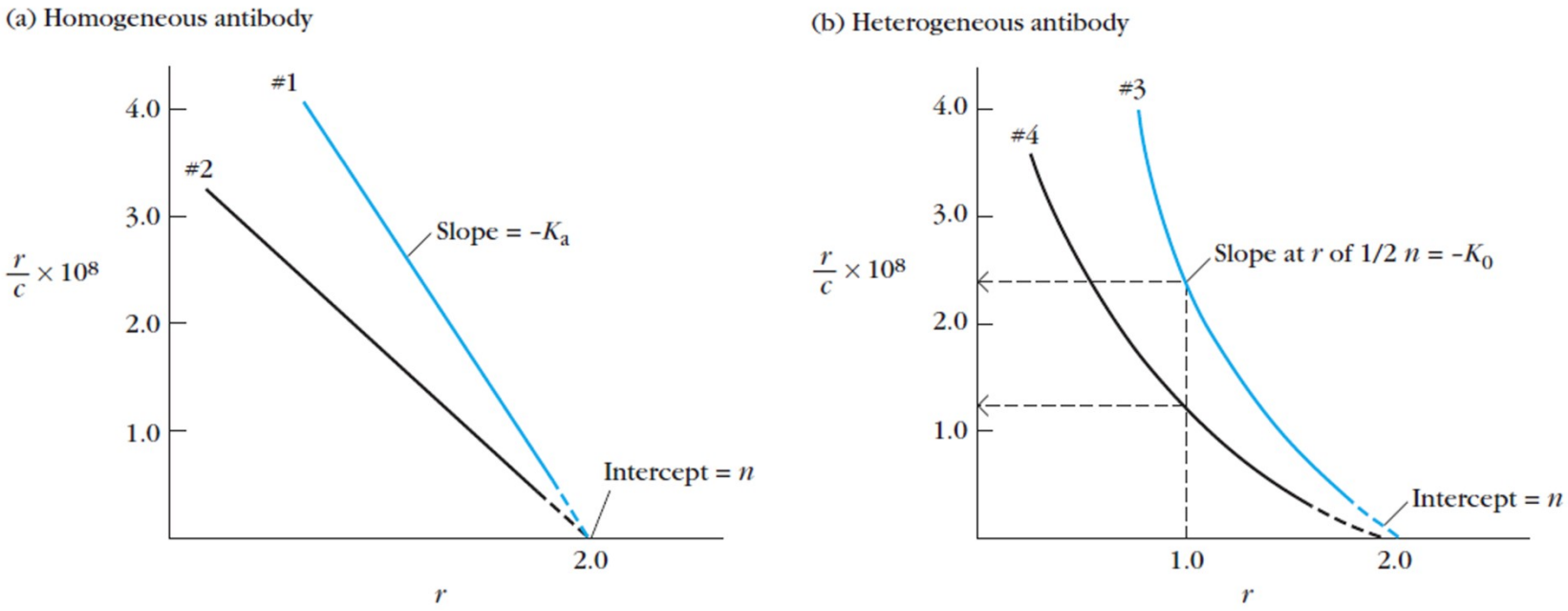
- where *k*1 is the forward (association) rate constant and *k*1 is the reverse (dissociation) rate constant. The ratio *k*1/*k*1 is the association constant *K*a (i.e., *k*1/*k*1 *K*a), a measure of affinity. Because *K*a is the equilibrium constant for the above reaction, it can be calculated from the ratio of the molar concentration of bound Ag-Ab complex to the molar concentrations of unbound antigen and antibody at equilibrium as follows:

$$K_a = \frac{[\mathrm{Ag\text{-}Ab}]}{[\mathrm{Ab}][\mathrm{Ag}]}$$

Antibody Affinity Is a Quantitative Measure of Binding Strength



Antibody Affinity Is a Quantitative Measure of Binding Strength



Antibody Avidity Incorporates Affinity of Multiple Binding Sites

- **The affinity at one binding site does not always reflect the true strength of the antibody-antigen interaction. When complex antigens containing multiple, repeating antigenic determinants are mixed with antibodies containing multiple binding sites, the interaction of an antibody molecule with an antigen molecule at one site will increase the probability of reaction between those two molecules at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the avidity.**

- **The avidity of an antibody is a better measure of its binding capacity within biological systems (e.g., the reaction of an antibody with antigenic determinants on a virus or bacterial cell) than the affinity of its individual binding sites. High avidity can compensate for low affinity. For example, secreted pentameric IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.**

Precipitation Reactions

- **Antibody and soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called precipitins. Although formation of the soluble Ag-Ab complex occurs within minutes, formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion.**

- **Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:**

- **The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.**

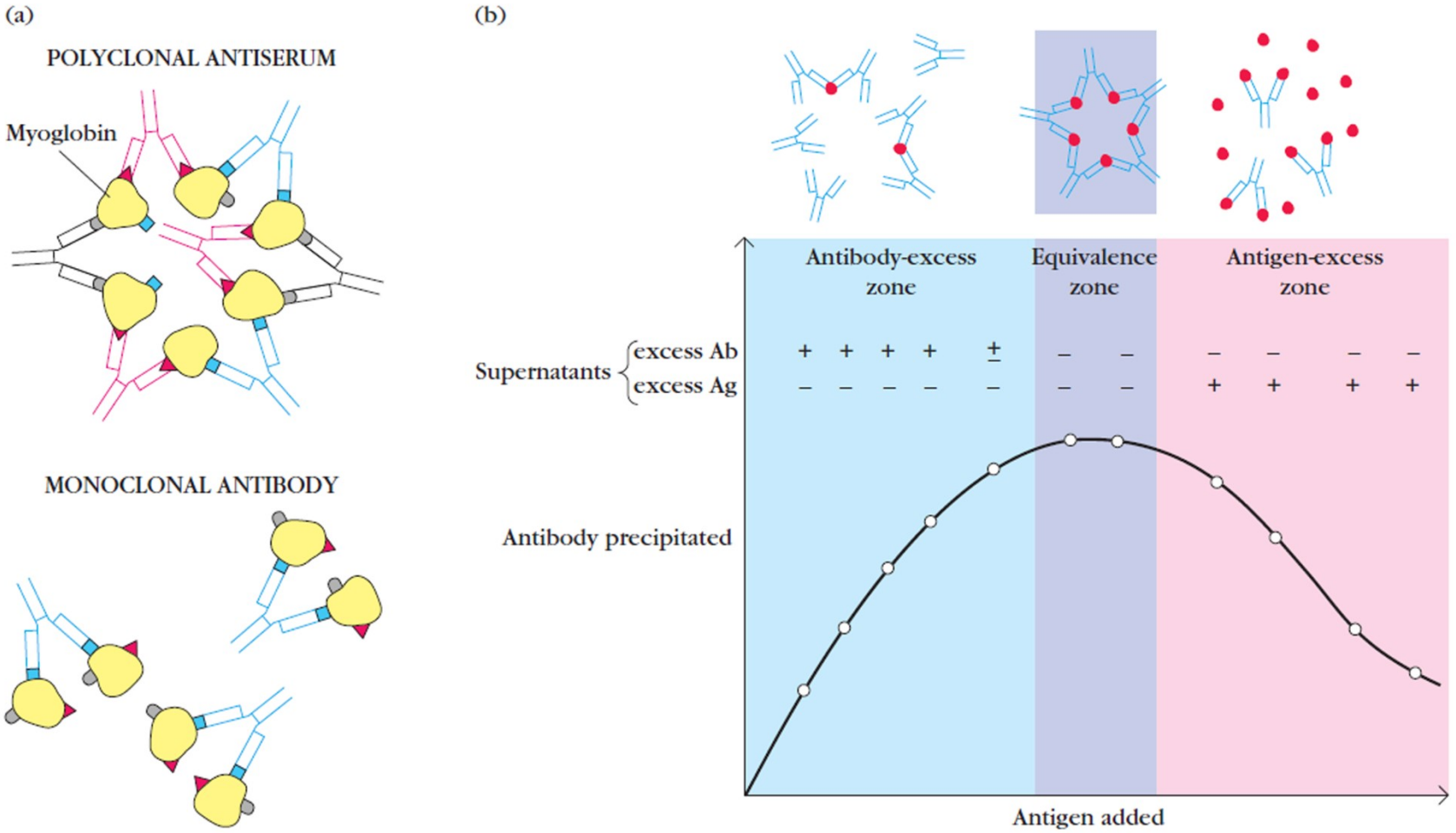
- **The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera.**

Precipitation Reactions

TABLE Sensitivity of various immunoassays

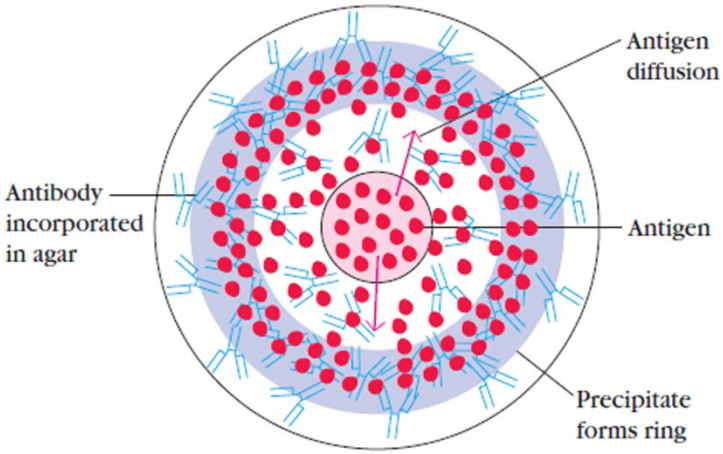
Assay	Sensitivity* (μg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reactions in gels	
Mancini radial immunodiffusion	10–50
Ouchterlony double immunodiffusion	20–200
Immunoelectrophoresis	20–200
Rocket electrophoresis	2
Agglutination reactions	
Direct	0.3
Passive agglutination	0.006–0.06
Agglutination inhibition	0.006–0.06
Radioimmunoassay	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	<0.0001–0.01
ELISA using chemiluminescence	<0.0001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.06–0.006

Precipitation Reactions in Fluids Yield a Precipitin Curve

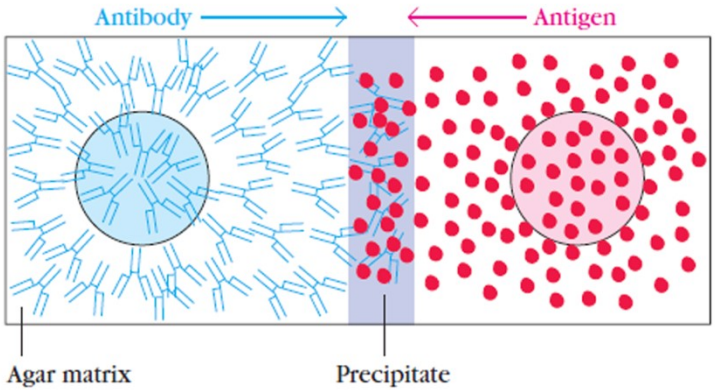


Precipitation Reactions in Gels Yield Visible Precipitin Lines

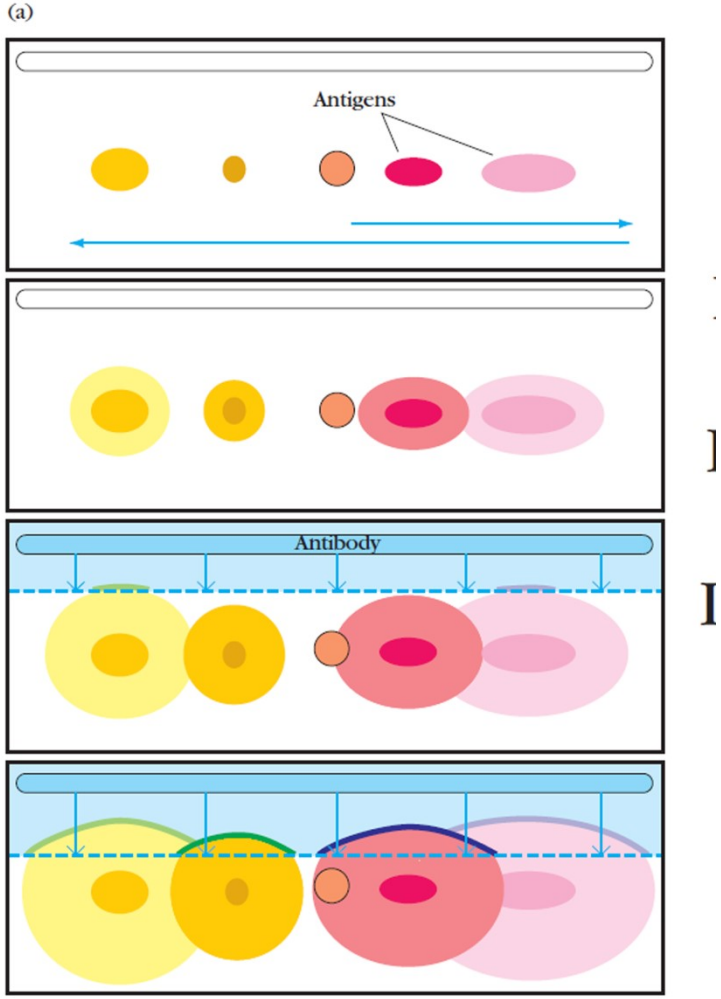
RADIAL IMMUNODIFFUSION



DOUBLE IMMUNODIFFUSION



Immunoelectrophoresis Combines Electrophoresis and Double Immunodiffusion

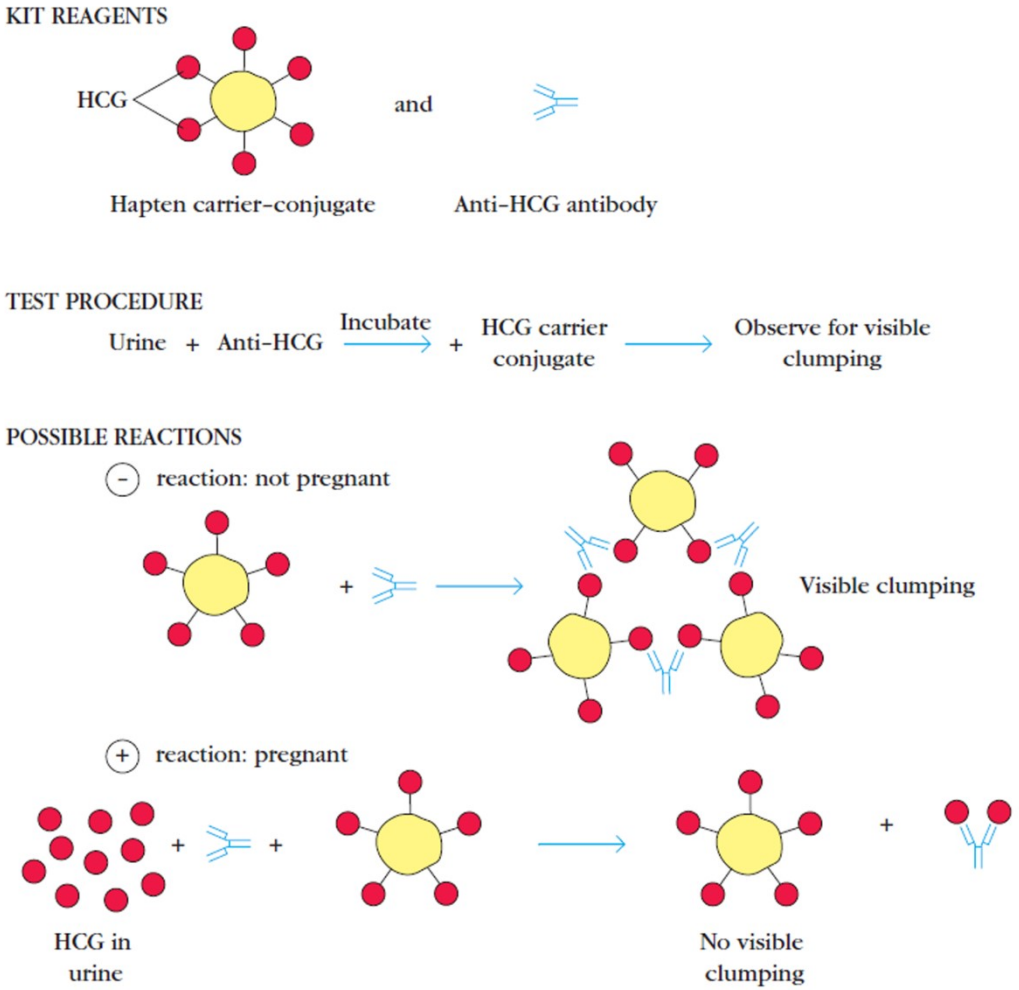


Agglutination Reactions

- **The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the crosslinking of polyvalent antigens.**

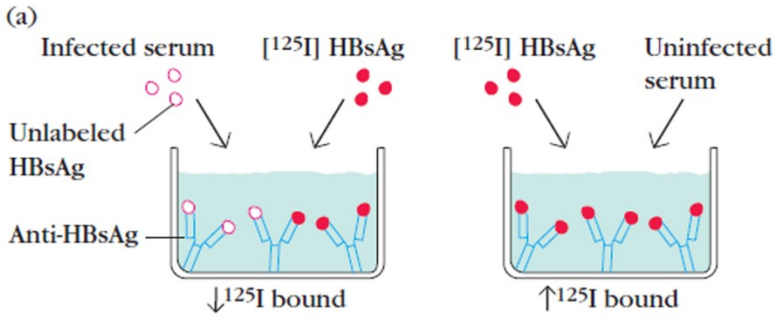
- **Agglutination reactions are routinely performed to type red blood cells (RBCs). In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.**

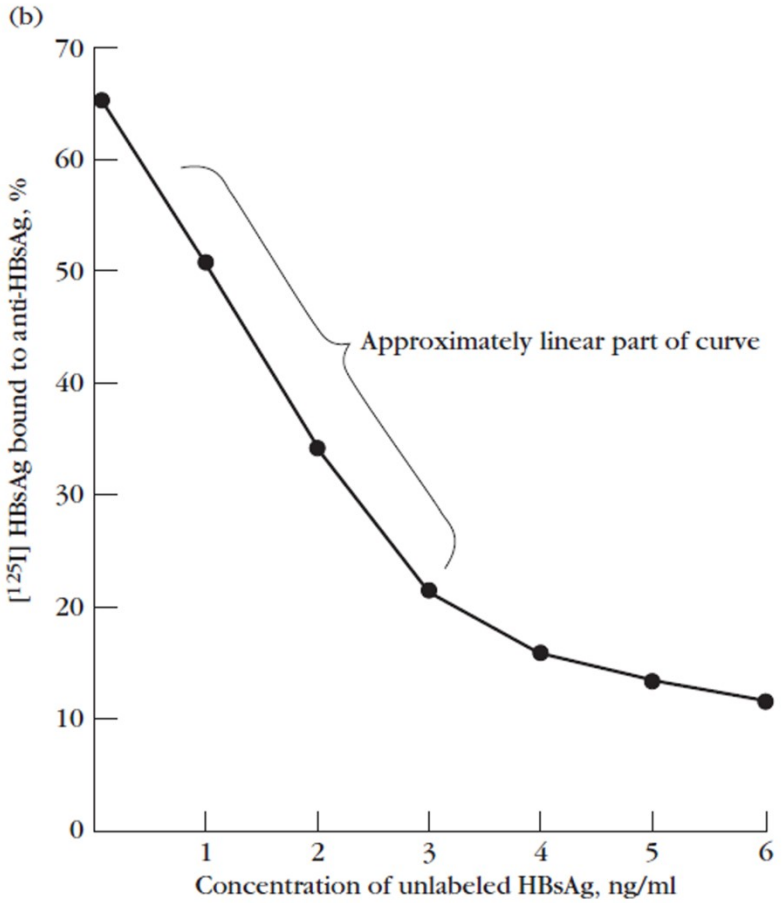
Agglutination Reactions



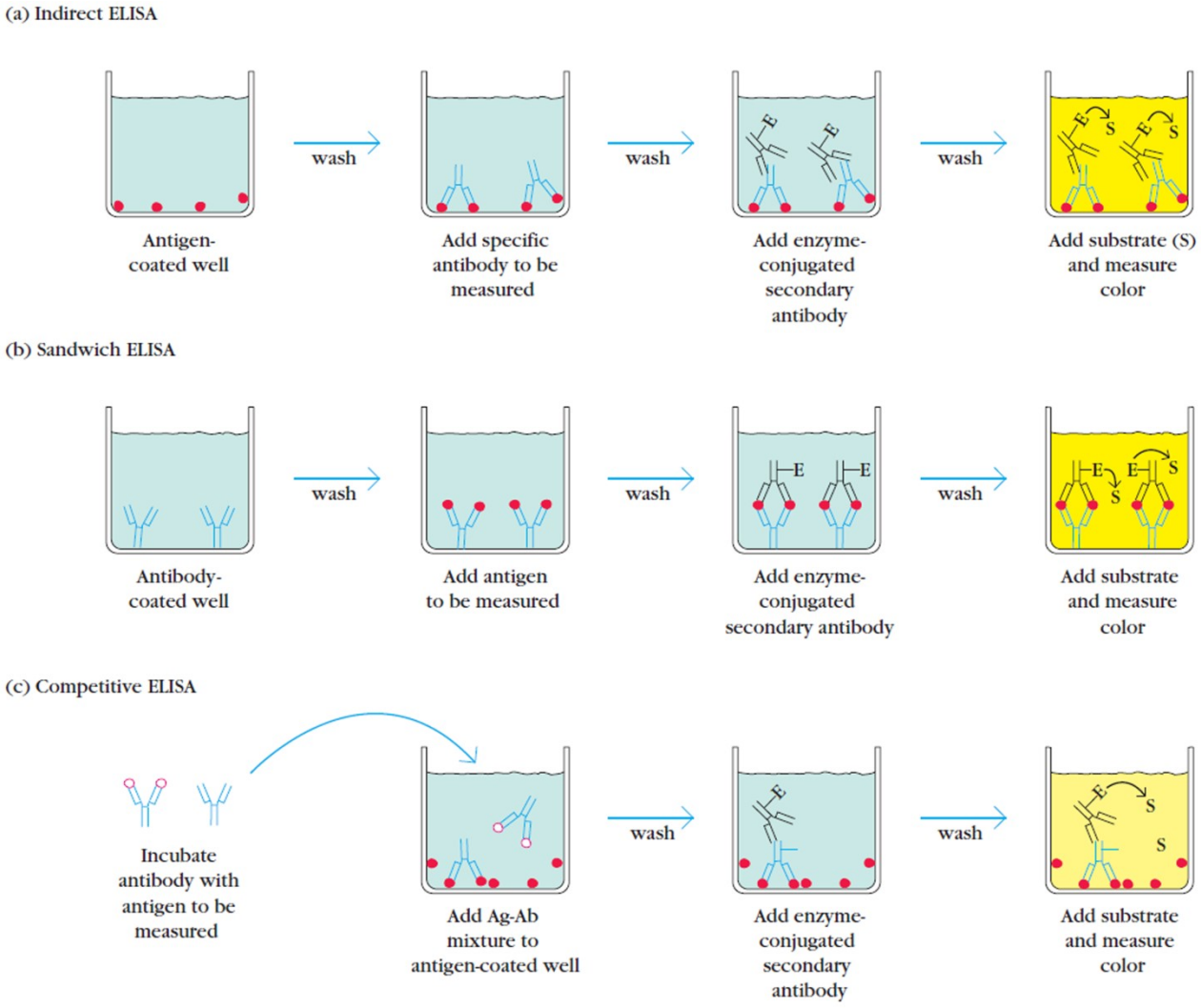
Radioimmunoassay

One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA).





Enzyme-Linked Immunosorbent Assay

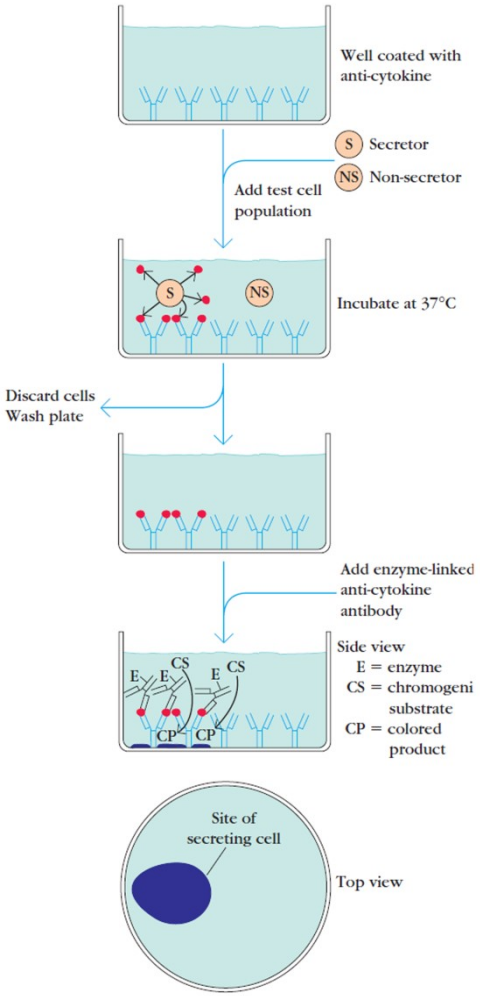


- **Enzyme-linked immunosorbent assay, commonly known as ELISA (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label.**

ELISPOT ASSAY

A modification of the ELISA assay called the ELISPOT assay allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen fo which one has a specific antibody

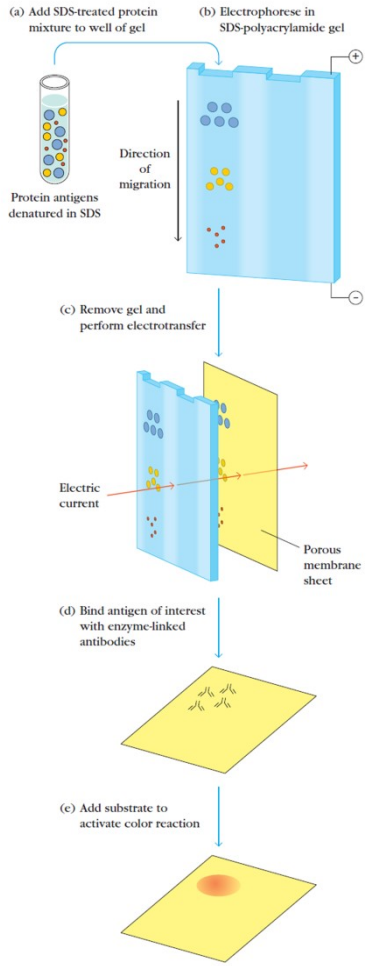
ELISPOT ASSAY



Western Blotting

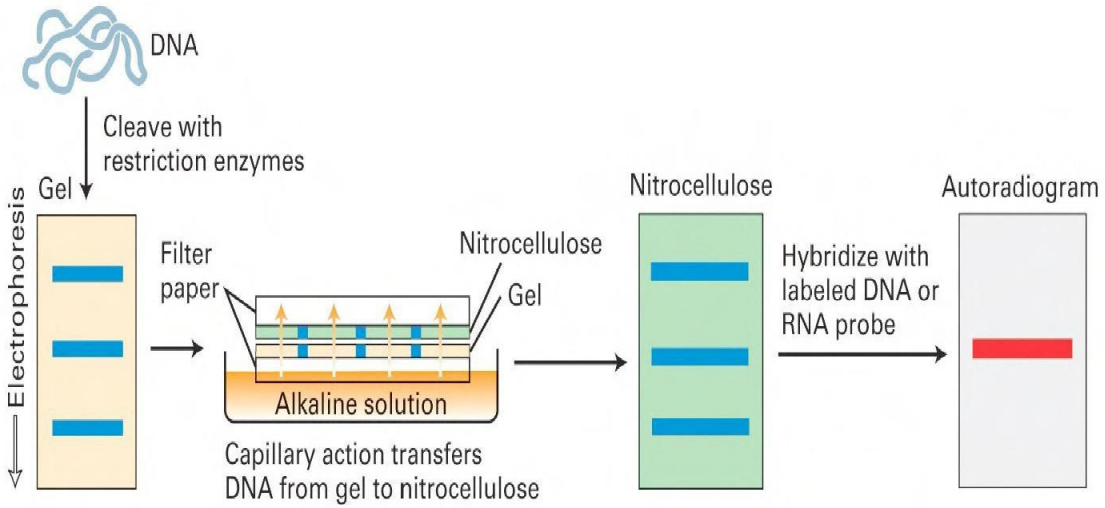
- **Identification of a specific protein in a complex mixture of proteins can be accomplished by a technique known as Western blotting, named for its similarity to Southern blotting, which detects DNA fragments, and Northern blotting, which detects mRNAs.**

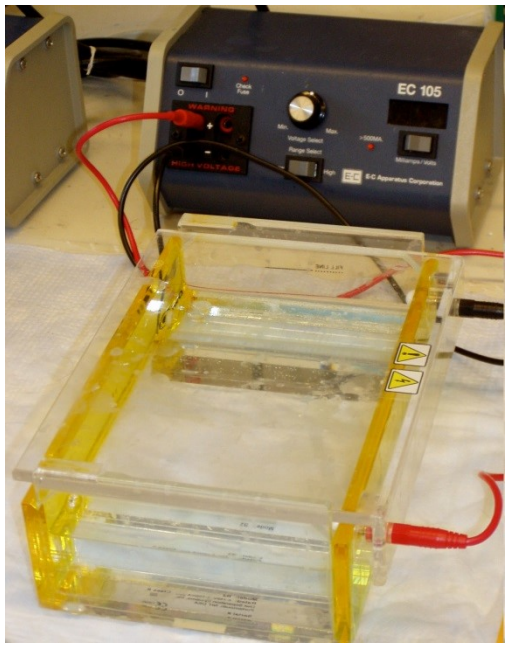
Western Blotting

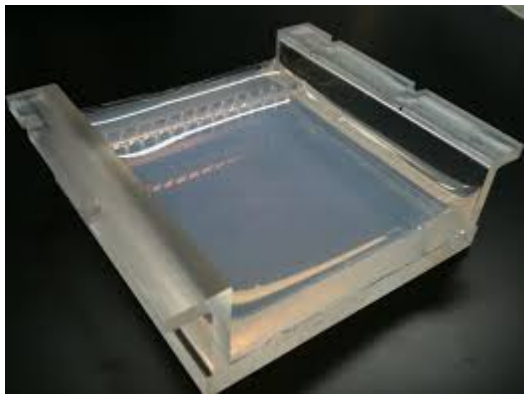


Mini Trans-Blot cell.

Southern Blotting







Southern Blotting

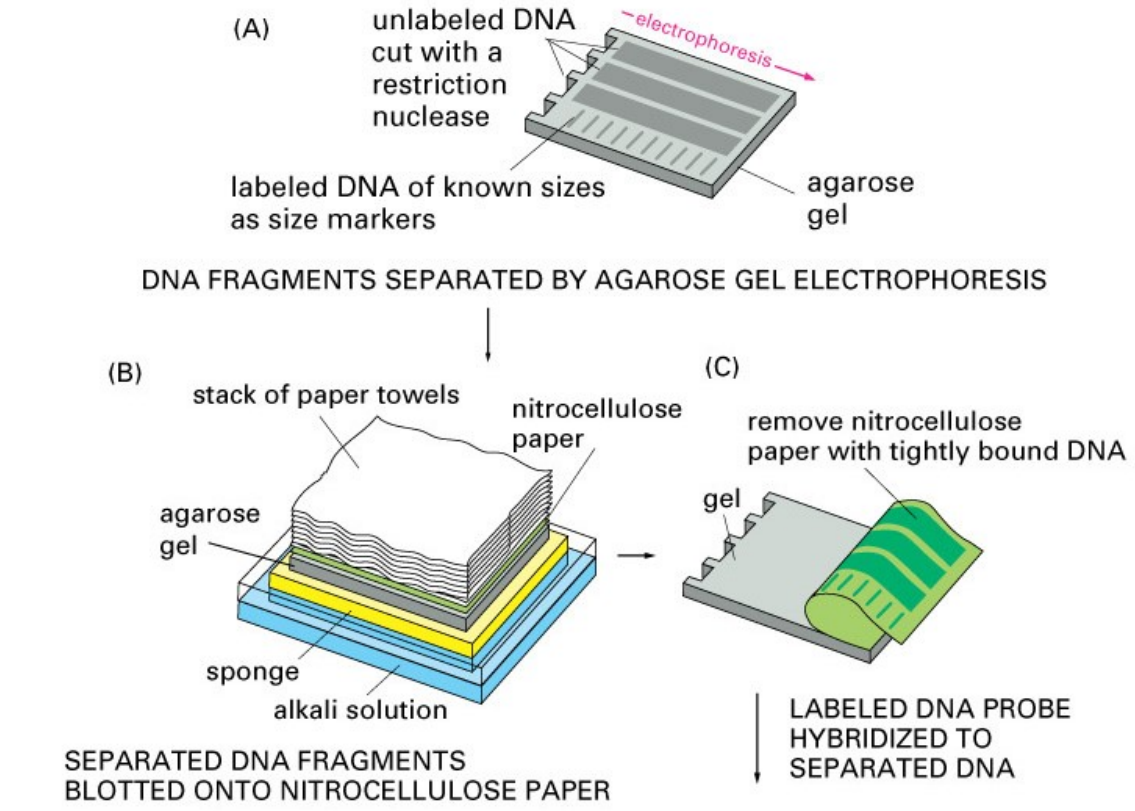
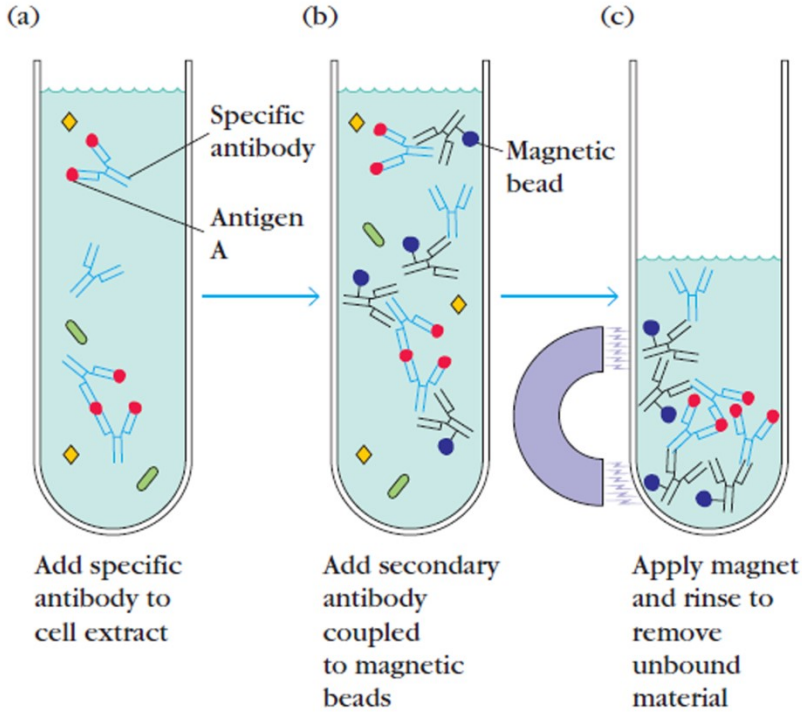


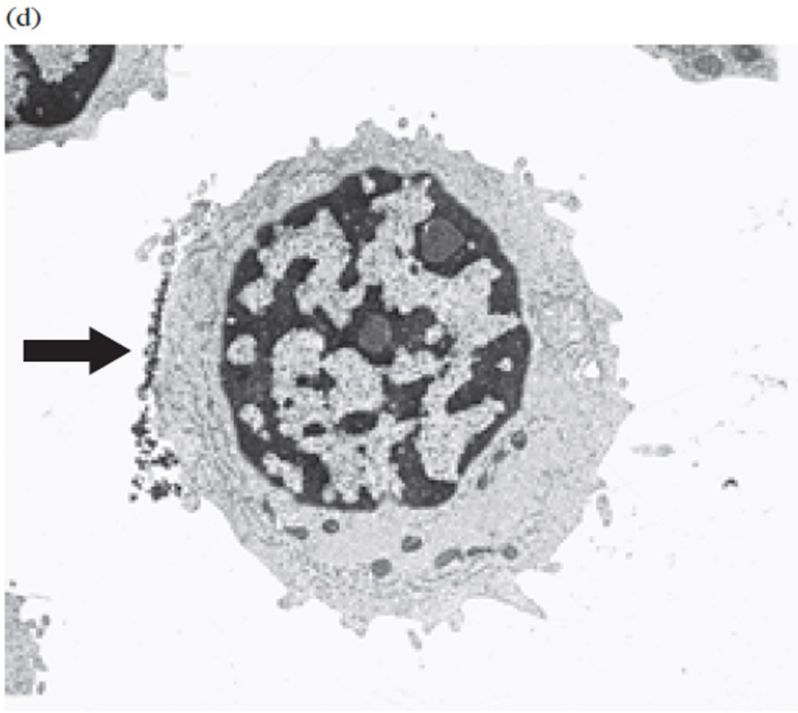
Figure 10-14 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Immunoprecipitation

- **The immunoprecipitation technique has the advantage of allowing the isolation of the antigen of interest for further analysis. It also provides a sensitive assay for the presence of a particular antigen in a given cell or tissue type. An extract produced by disruption of cells or tissues is mixed with an antibody against the antigen of interest in order to form an antigen-antibody complex that will precipitate. However, if the antigen concentration is low (often the case in cell and tissue extracts), the assembly of antigen-antibody complexes into precipitates can take hours, even days, and it is difficult to isolate the small amount of immunoprecipitate that forms.**

Immunoprecipitation



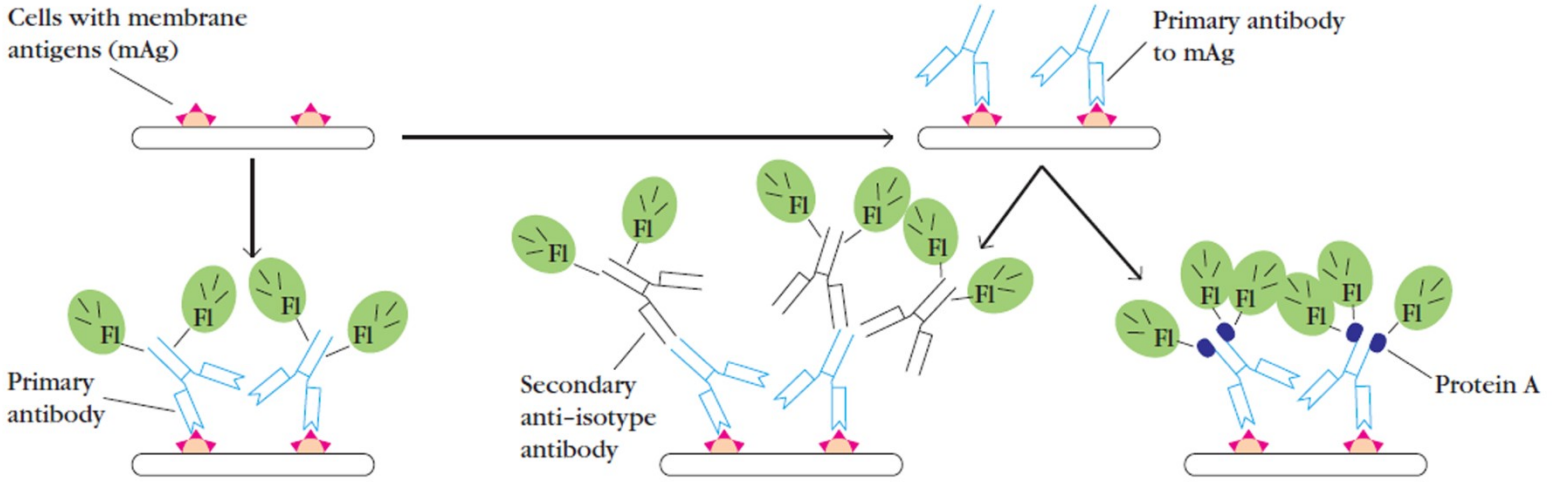


Immunofluorescence

- In 1944, Albert Coons showed that antibodies could be labeled with molecules that have the property of fluorescence. Fluorescent molecules absorb light of one wavelength (excitation) and emit light of another wavelength (emission).

- If antibody molecules are tagged with a fluorescent dye, or fluorochrome, immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength.

Immunofluorescence

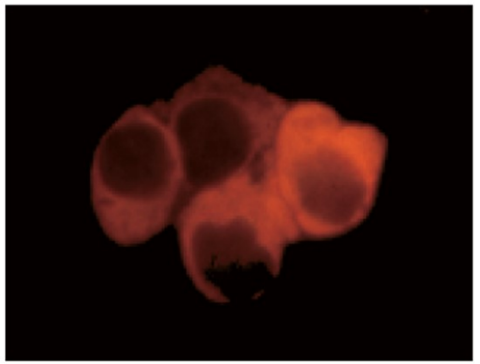


(a) Direct method with fluorochrome-labeled antibody to mAg

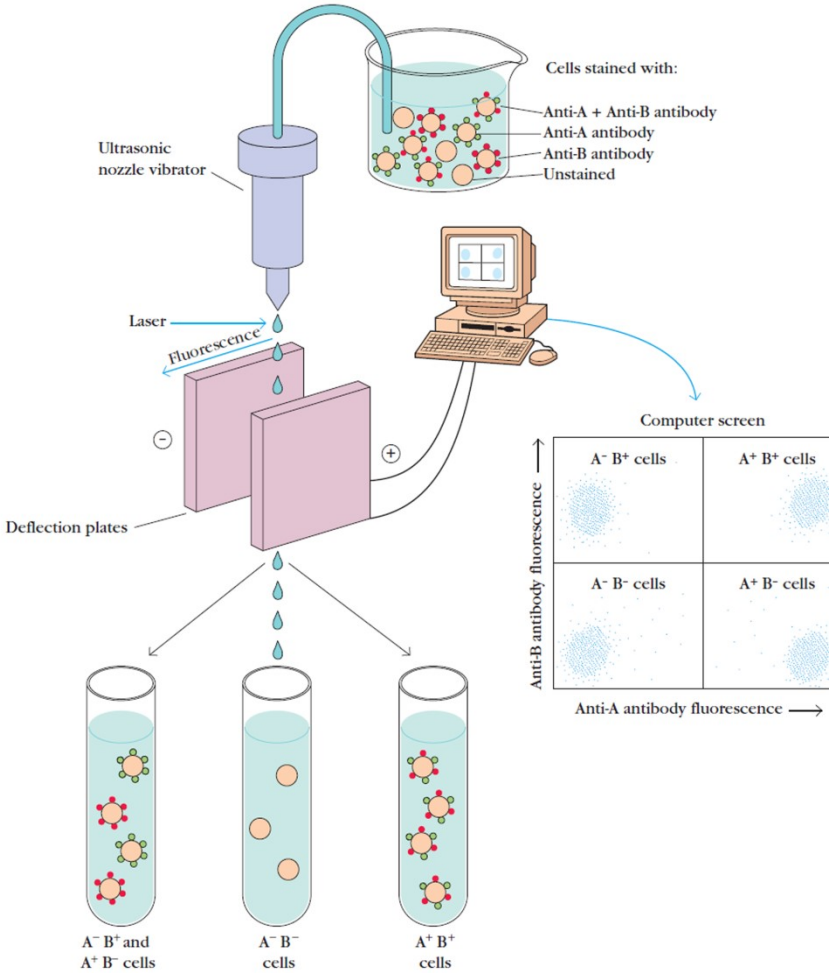
(b) Indirect method with fluorochrome-labeled anti-isotype antibody

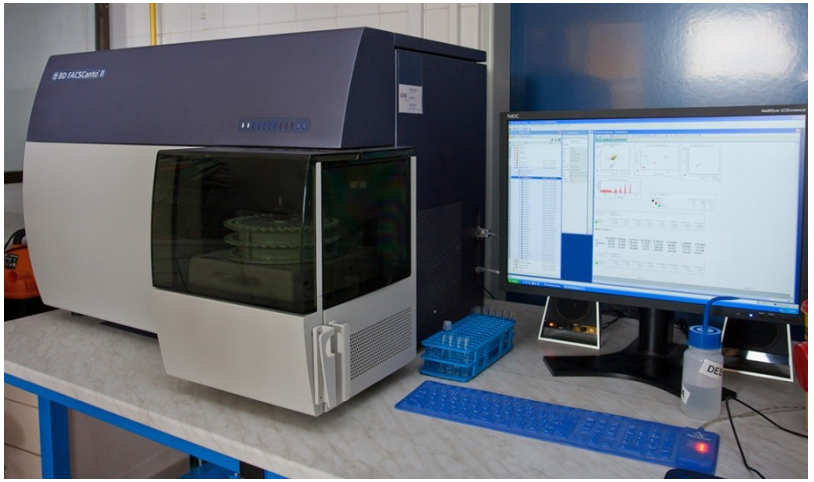
(c) Indirect method with fluorochrome-labeled protein A

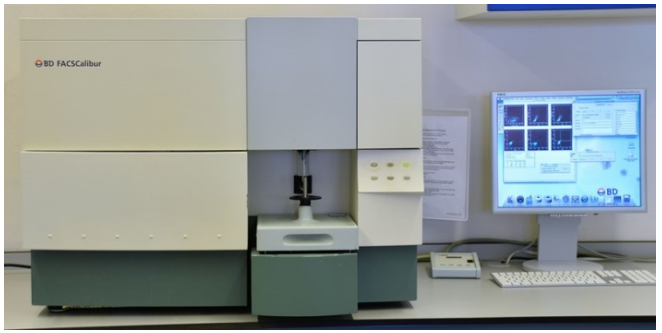
(d)



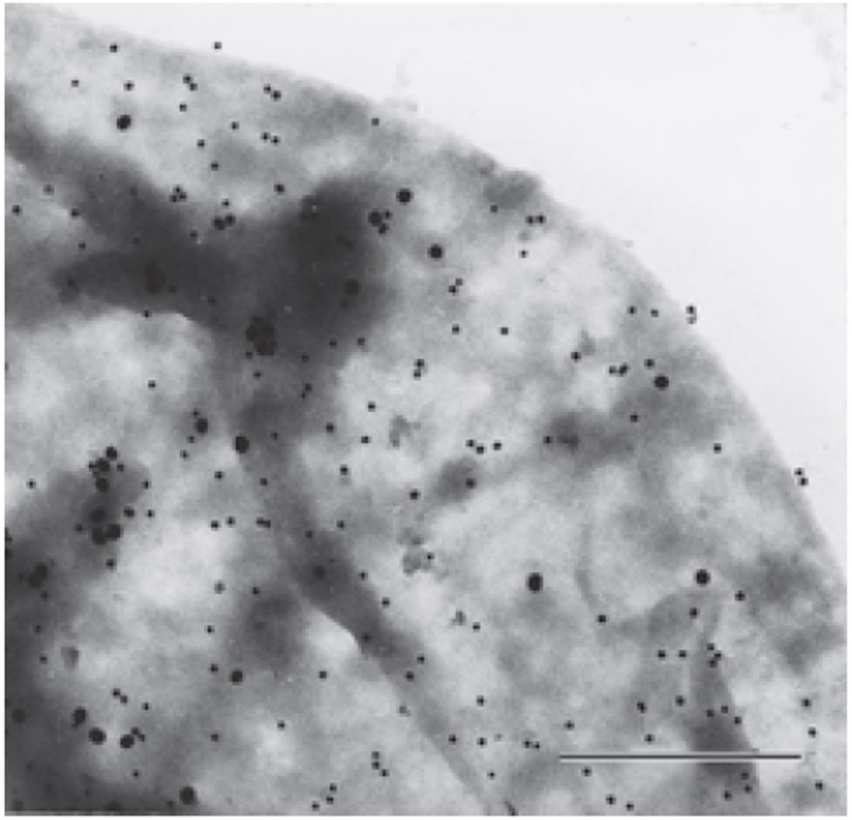
Flow Cytometry and Fluorescence







Immunoelectron Microscopy

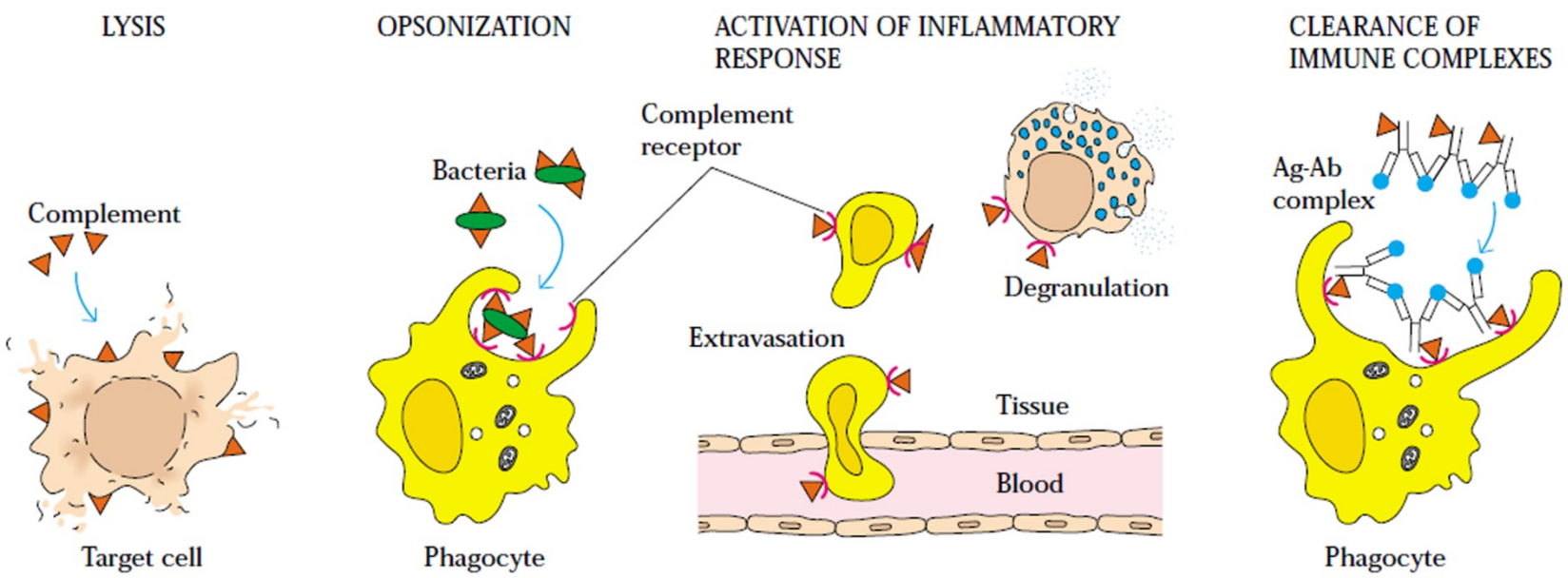


The fine specificity of antibodies has made them powerful tools for visualizing specific intracellular tissue componentsby **immunoelectron microscopy.** In this technique, an electron-dense label is either conjugated to the Fc portion of a specific antibody for direct staining or conjugated to an antiimmunoglobulin reagent for indirect staining. A number of electron-dense labels have been employed, including *ferritin* and *colloidal gold.* Because the electron-dense label absorbs electrons, it can be visualized with the electron microscope as small black dots. In the case of immunogold labeling, different antibodies can be conjugated with gold particles of different sizes, allowing identification of several antigens within a

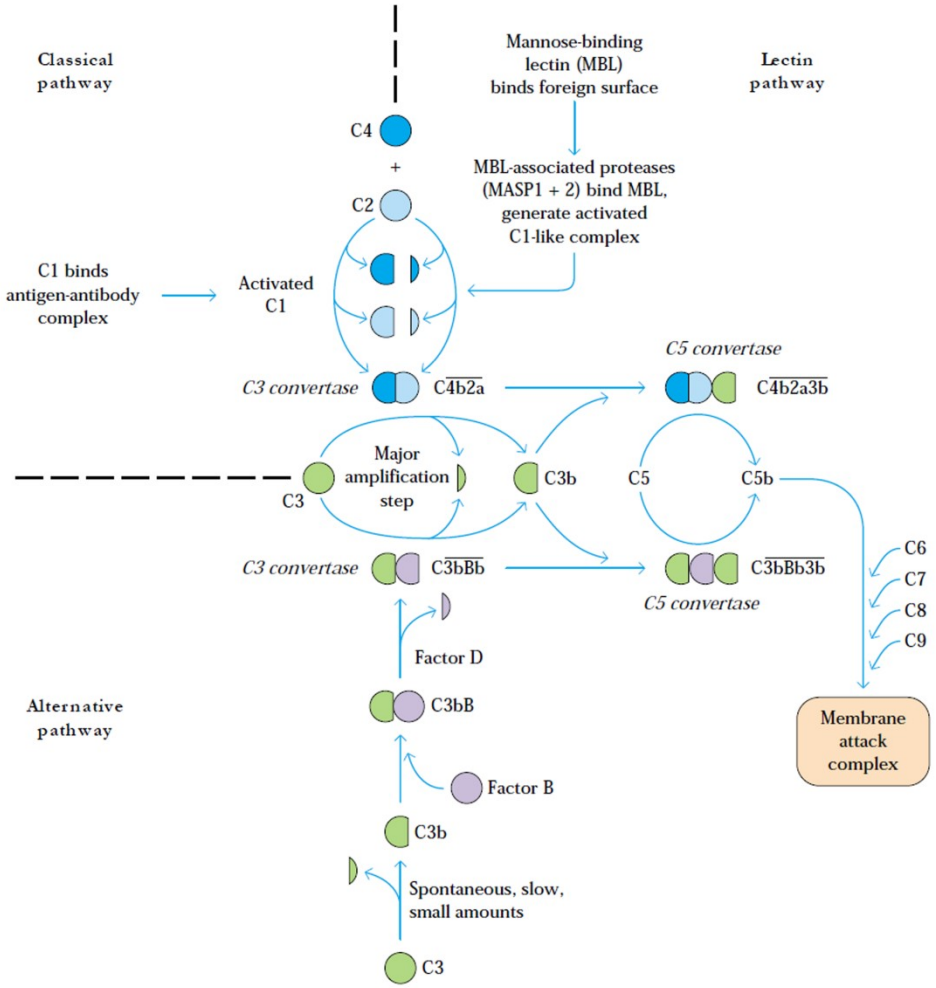
The Complement System

- **The complement system is the major effector of the humoral branch of the immune system.**

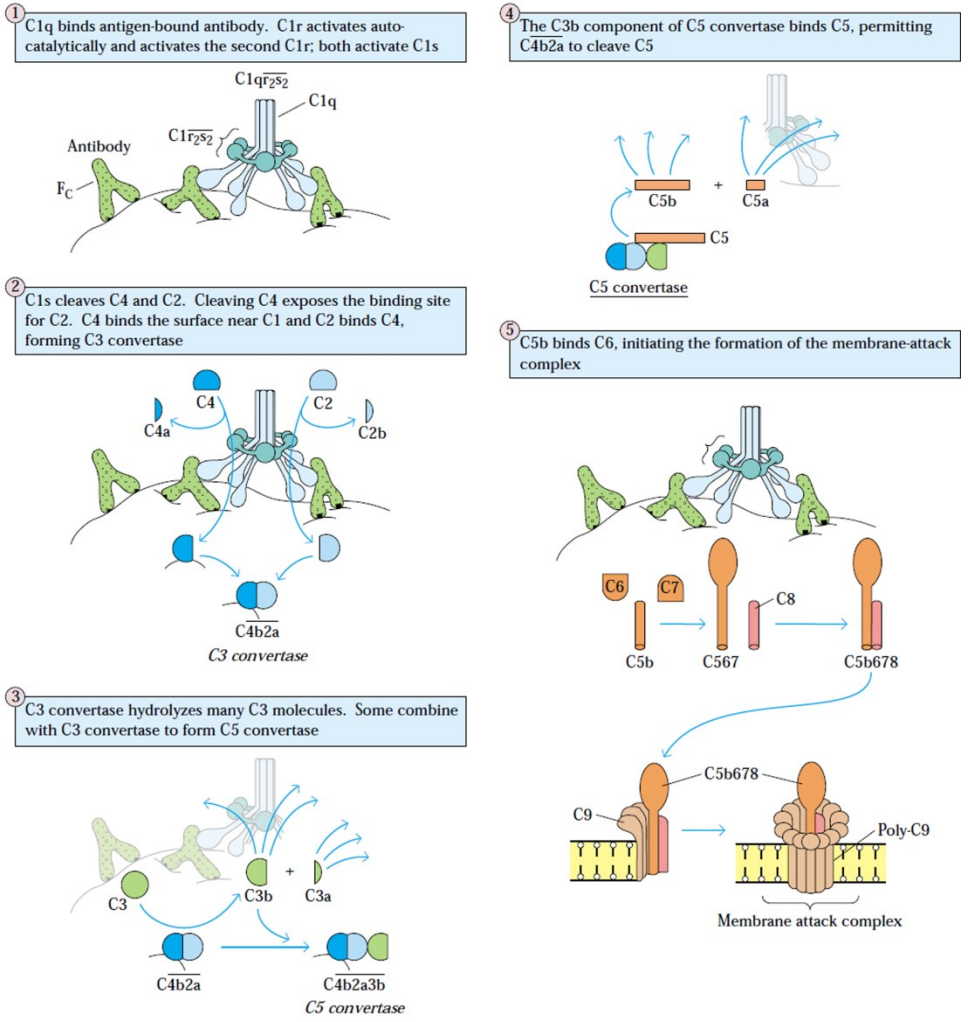
The Functions of Complement



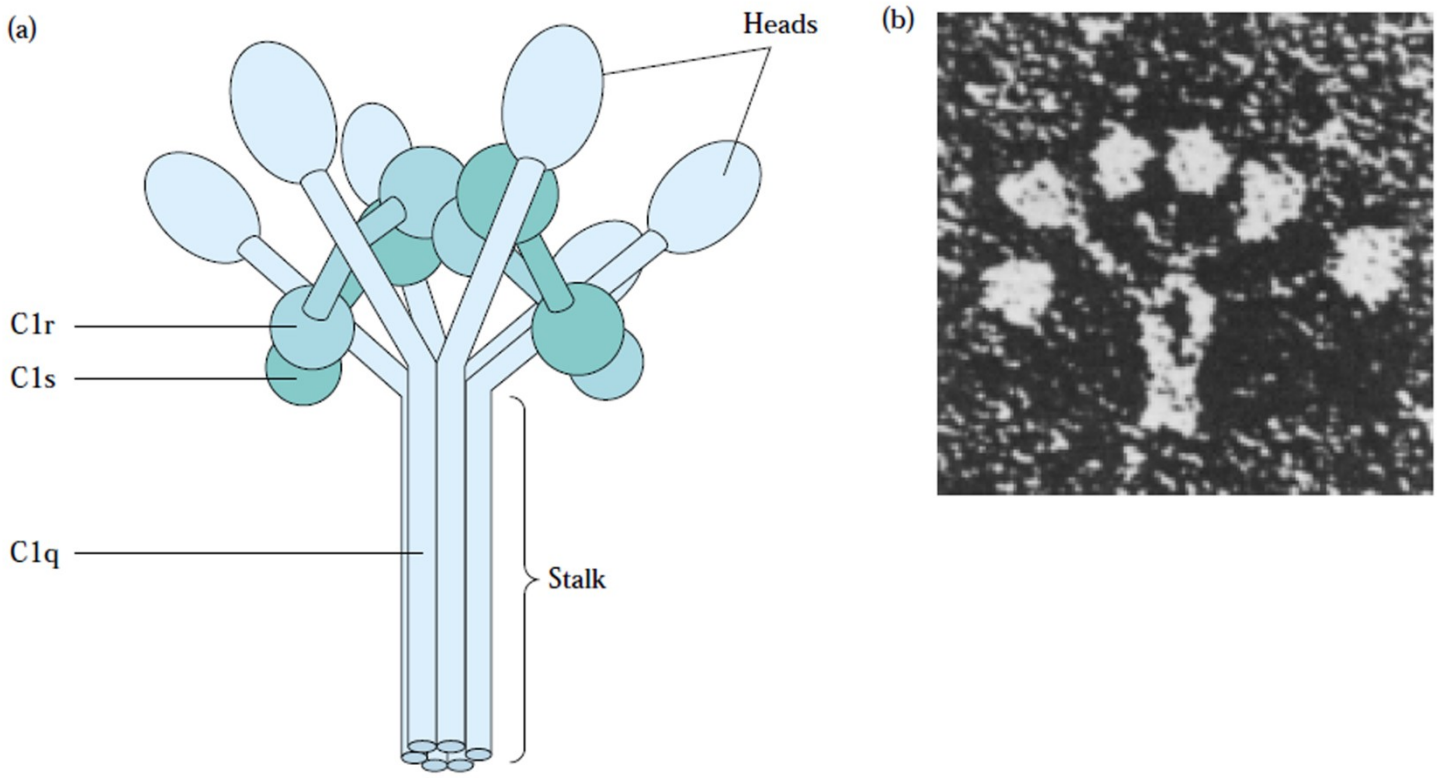
The Classical Pathway Begins with Antigen-Antibody Binding



The Classical Pathway Begins with Antigen-Antibody Binding



The Classical Pathway Begins with Antigen-Antibody Binding



Structure of the C1 macromolecular complex.

The Alternative Pathway Is Antibody-Independent

TABLE Initiators of the alternative pathway of complement activation

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)
NONPATHOGENS
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)

The Alternative Pathway Is Antibody-Independent