IMMUNOLOGY

THE IMMUNE SYSTEM	ONTOGENTY OF IMMUNE SYSTEM	LYMPHOCYTE DEVLOPMENT AND SELECTION	PHERIPHERY : INNATE IMMUNE RESPONSE	SECONDARY LYMPHOID TISSUE : INNATE IMMUNE RESPONSE MEETS ADAPTIVE
SOCONDARY LYMPHOID TISSUE: T AND B CELL ACTIVATION	HUMORAL IMMUNITY	CELL MEDIATEDD IMMUNITY	IMMUNODIAGNOSIS	IMMUNIZATIONS
	PRIMARY IMMUNE DEFICIENCY	HYPERSENSITIVITY	TRANSPLANTATION	

Immunology

IMMUNOLOGY

IMMUNODIGNOSIS

IMMUNODIGNOSIS

Learning Objectives

- Describe the recirculation pattern of memory B and T lymphocytes
- Differentiate between primary and secondary immune response

IMMUNODIAGNOSIS

SEROLOGY

Serology is an important diagnostic tool for many diseases including infections and autoimmune disorders. The interaction of antigen and antibody that occurs in vivo and in clinical laboratory settings provides the basis for all serologically based tests.

IMMUNODIAGNOSIS

IgM and IgG

IgM is the principal immunoglobulin of the primary immune response when antigen is first encountered. It is replaced in later responses by antibodies of different isotypes, mostly IgG in the serum.

IgM antibodies are occasionally produced at low levels during secondary and later immunologic responses, they are always produced by cells encountering that antigen for the first time.

IgM is extremely important in diagnosis of recent infections and infections in neonates or foetus. For example, a patient with IgM antibodies to the core antigen of HBV (HBcAb) is an important diagnostic tool because it suggests a recent or acute infection and may also be found in the window period when other antibodies maynot be detectable.

Also, we can make certain assumptions based on serology using IgM in the diagnoses of neonatal or fetal infections. For example, a neonate that is making IgM specific for a virus such as rubella is infected with the virus rather than immune or protected by maternal antibodies. This is because IgM does not cross the placenta.Therefore, the only way a neonate or foetus can be producing IgM specific for a certain pathogen is if the neonate or foetus were infected with that agent. The predominant isotype of immunoglobulin that begins to be produced after IgM during the primary immune response is IgG.

IMMUNODIAGNOSIS

Primary and secondary antibodies response

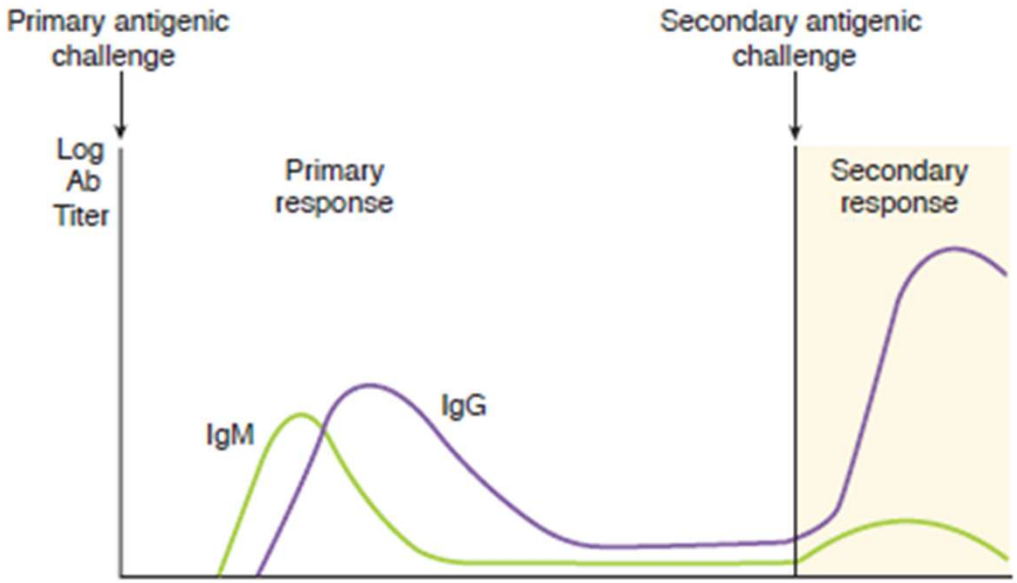


Figure I-9-1. Primary and Secondary Antibody Responses

IMMUNODIAGNOSIS

Ideotype, Isotype, and Allotype

The unique pocket created by the variable regions of the light chain and the heavy chain is called the **idiotype** of the antibody. It is the region that is specific for antigen. It is both extremely diverse and specific. Each individual is capable of producing hundreds of millions of unique idiotypes.

The **isotype** of the antibody is determined by the constant region and is encoded by the heavy chain genes. The isotype of the antibody determines its function.

The **allotype** of an antibody is an allelic difference in the same antibody isotypes that differ between people. For example, 2 individuals with the same IgG have subtle differences in their immunoglobulins due to heterogeneity which tends to be specific for individuals. A patient receiving pooled gamma globulins might react to these allotypic differences in the constant regions which may result in type III hypersensitivity reactions.

IMMUNODIAGNOSIS

Membrane Bound Immunoglobulin

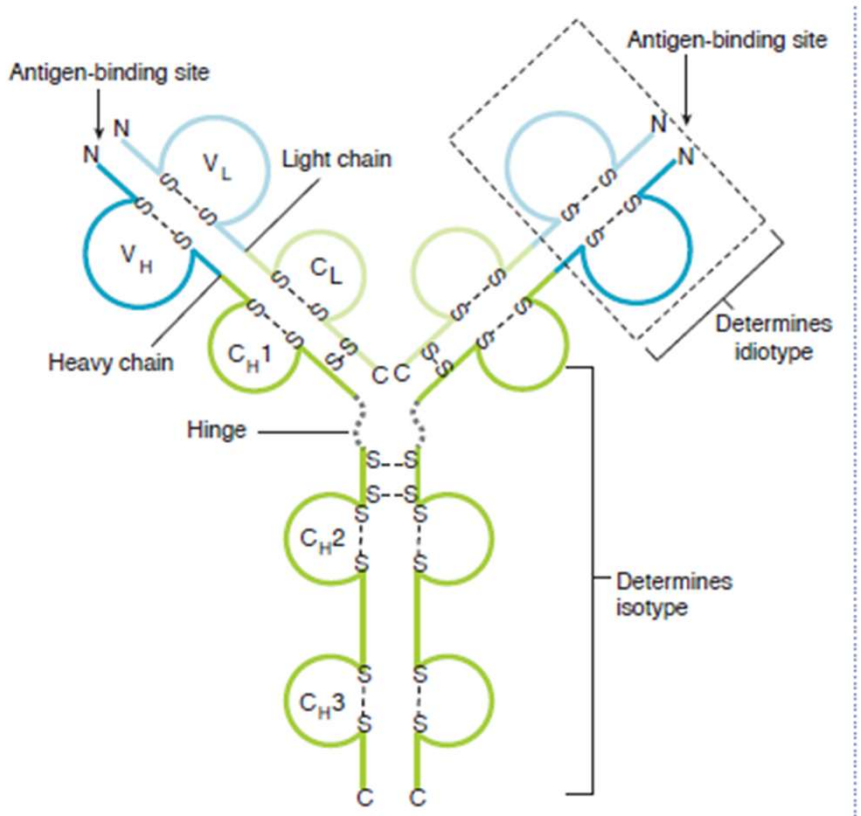


Figure I-9-2. B-Lymphocyte Antigen Recognition Molecule (Membrane-Bound Immunoglobulin)

IMMUNODIAGNOSIS

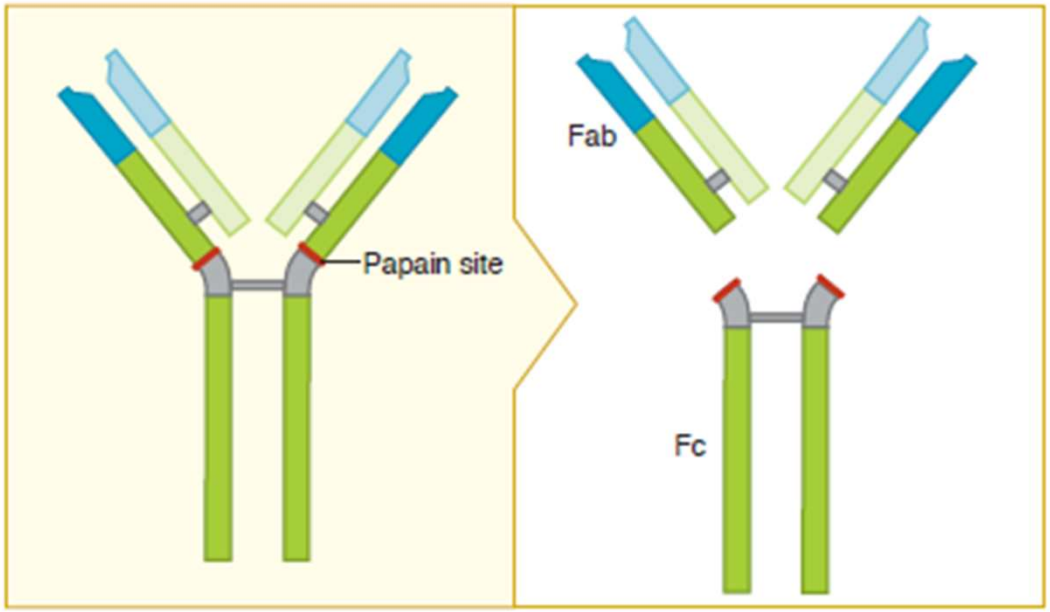
Papain versus Pepsin Digestion

The biologic function of segments of the antibody molecule was fi st elucidated by digestion of these molecules with proteolytic enzymes. If an antibody molecule is digested with papain, cleavage occurs above the disulphide bonds that hold the heavy chains together. Th s generates 3 separate fragments, two of which are called Fab (fragment antigen binding), and one of which is called Fc (fragment crystallizable).

Cleavage of the antibody molecule with pepsin generates one large fragment called F(ab´)2 and a digested Fc fragment. The bridging of antigens by antibody molecules is required for agglutination of particulate antigens or the precipitation of soluble antigens.

IMMUNODIAGNOSIS

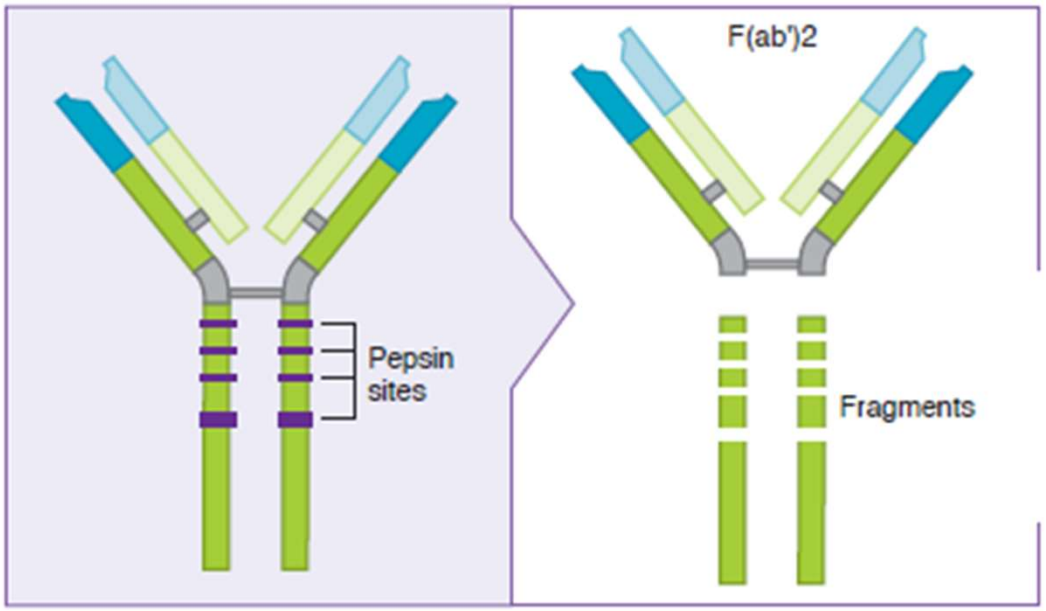
Proteolytic Cleavage with Papain



Proteolytic Cleavage with Papain

IMMUNODIAGNOSIS

Proteolytic cleavage by Pepsin



Proteolytic Cleavage with Pepsin

IMMUNODIAGNOSIS

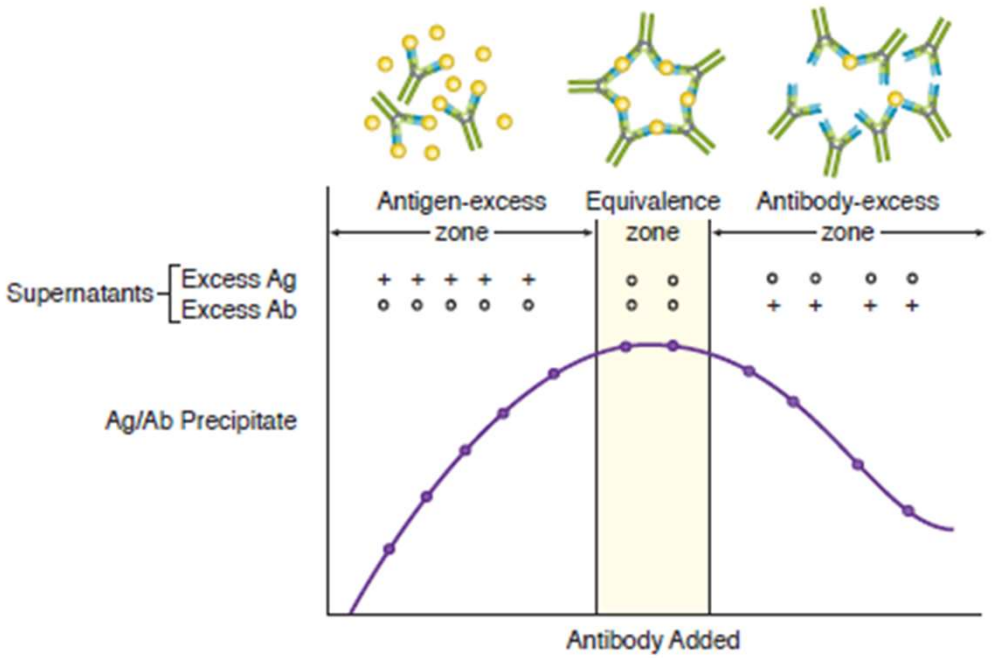
Zone of Equivalence

Interaction of antigen and antibody occurs in vivo, and in clinical settings it provides the basis for all serologically based assays. The formation of immune complexes produces a visible reaction that is the basis of precipitation and agglutination assays. Agglutination and precipitation are maximized when multiple antibody molecules share the binding of multiple antigenic determinants, a condition known as **equivalence**.

In vivo, the precipitation of such complexes from the blood is critical to the trapping of pathogens and the initiation of the immune response in the secondary lymphoid organs, as well as the initiation of the pathologic phase of many immune complex-mediated diseases. In vitro, the kinetics of such reactions can be observed by titration of antigen against its specific antibody.

IMMUNODIAGNOSIS

Zone of equivalence



IMMUNODIAGNOSIS

Normal progression of the antibody response during many infectious diseases.

- At the **start of the infection**, the patient is in a state of antigen excess; the pathogen is proliferating in the host and the development of specific antibodies has not yet begun.
- As the patient begins to make an **adequate antibody response**, he enters the equivalence zone; all available antigen is complexed with antibody, and neither free antigen nor free antibody can be detected in the serum.
- Finally, as the **infection is resolved**, the patient enters the antibody excess zone, when more antibody is being produced than is necessary to precipitate all available antigen.

IMMUNODIAGNOSIS

The clinical demonstration of this phenomenon is most easily seen in our use of the serologic diagnosis of hepatitis B infection.

• Early in the course of this infection, HBsAg is easily detectable in the blood. The patient is in the antigen excess zone for this antigen.

• As the patient enters the window period (the equivalence zone), the HBsAg disappears from the circulation because it is being removed by antibody precipitation.

• Finally, antibody titres (HBsAb) rise in the serum as the patient enters the antibody excess zone and resolves the infection. Although the “window period” in the hepatitis B infection is used exclusively to note the absence of HBsAg and HBsAb from the serum (only antigen–antibody response that has a clinical significance in the prognosis of disease). An equivalence zone is a universal stage in the development of any antibody–antigen interaction.

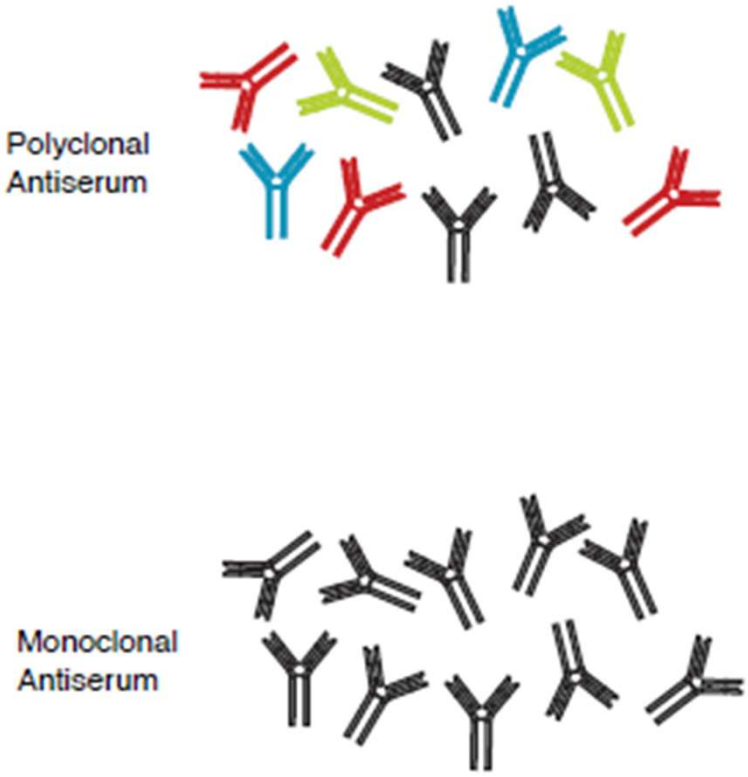
IMMUNODIAGNOSIS

Monoclonal versus Polyclonal Antiserum

Polyclonal antiserum is generally produced in an individual naturally during any type of infection. It represents many different clones of B cells that are making antibodies to many different epitopes on an antigen; therefore a heterogenous complex mixture of antibodies is produced. Alternatively, polyclonal antiserum can be produced by inoculating an animal such as a mouse, rabbit or goat. Th s is done to produce commercial antiserum that can be purchased and utilized in laboratories. Monoclonal antibodies are produced by one clone of B cells with specific ty for the exact same epitope on an antigen. Monoclonal antibodies are produced in the laboratory and are used in all aspects of medicine from diagnostics to treatments for various types of cancer and autoimmune diseases.

IMMUNODIAGNOSIS

Monoclonal vs Polyclonal Antibodies



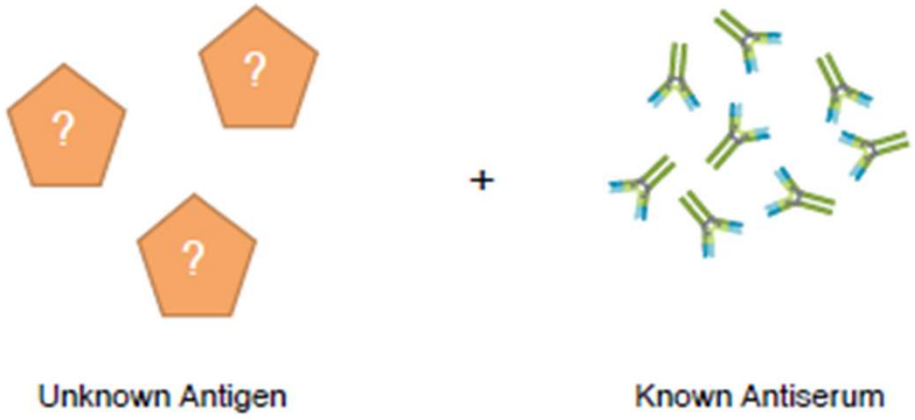
IMMUNODIAGNOSIS

Direct Serological tests

Direct serologic testing utilizes a known antiserum in order to detect an unknown antigen, either foreign or self. Direct tests are qualitative and provide results relatively quickly. They are used mostly for screening purposes.

IMMUNODIAGNOSIS

Direct Serological Test



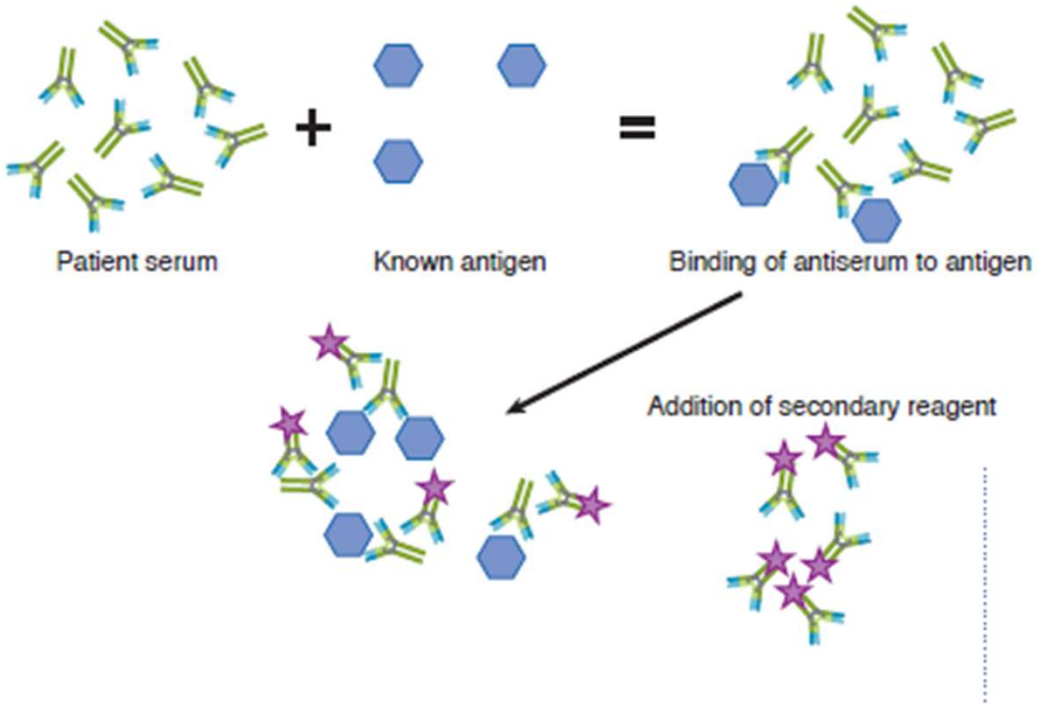
IMMUNODIAGNOSIS

Indirect Serological Test

- Indirect serologic testing utilizes antibodies from the patient that may be specific for either self or foreign antigen. Th s test is based on the concept that antibodies are produced in response to a specific disease state. Indirect tests may be qualitative and used for screening purposes or quantitative, which provides the amount of antibody in the patient's serum. Quantitative tests are also called antibody titres.
- A titre is often done to follow the progression of disease in a patient by looking for an increase or decrease in the level of antibodies. A titre involves diluting the patient's serum out to see how dilute the serum can be and still detect antigens in a solution.
- Most immunologic tests can be performed using direct or indirect measures. Indirect tests are generally more specific, resulting in fewer false-positives. The Coombs, ELISA, and fluorescent antibody tests are all examples of tests that can be utilized in either a direct or indirect manner.

IMMUNODIAGNOSIS

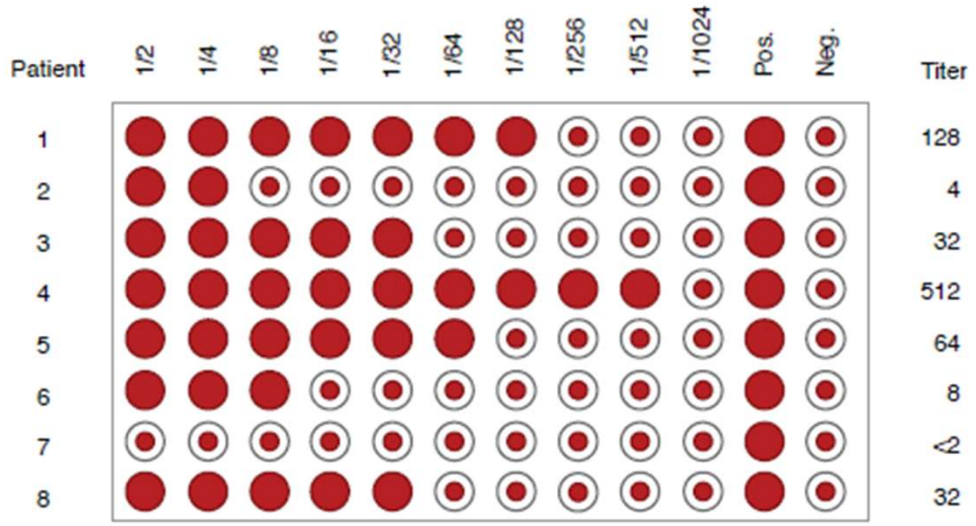
Indirect Serological Test



IMMUNODIAGNOSIS

Indirect Serological Test

Titration



IMMUNODIAGNOSIS

AGGLUTINATION

Agglutination tests are widespread in clinical medicine and are simply a variationon precipitation reactions. In agglutination reactions, the antigen is a particulate antigen such as RBCs or latex beads. Both will clump up to form of a lattice of antibody-bound particles in the presence of appropriate antibodies.

- Latex bead agglutination tests are available for the diagnosis of cerebrospinal infections such as *Haemophilus, pneumococcus, meningococcus, and Cryptococcus*. In each of these cases, antibodies against these organisms are conjugated to latex beads, and the presence of microbial antigens in the CSF is detected by the subsequent agglutination of those beads.
- RBC agglutination reactions are important in defining ABO blood groups, diagnosing Epstein-Barr virus infection (the monospot test), and identifying Coombs test for Rh incompatibility.

IMMUNODIAGNOSIS

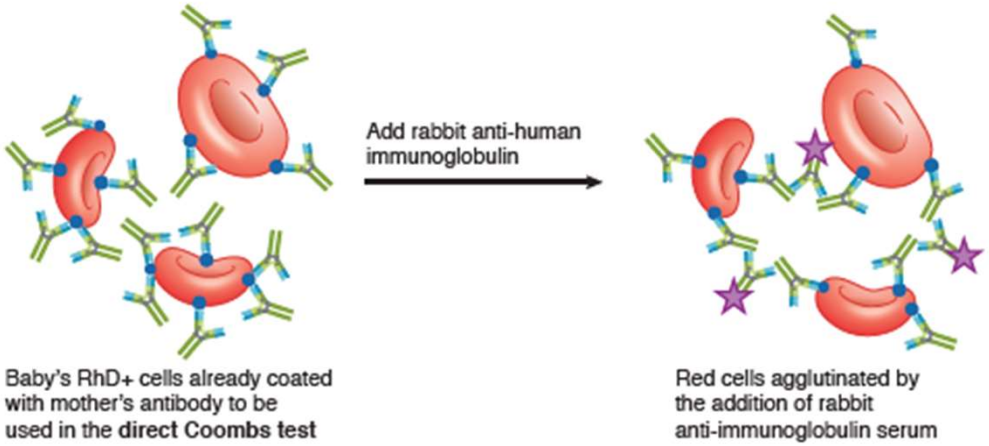
Coombs Test

Two variations of the Coombs test exist.

The **Direct Coombs** is designed to identify maternal anti-Rh antibodies that are already bound to infant RBCs or antibodies bound to RBCs in patients with autoimmune haemolytic anaemia.

IMMUNODIAGNOSIS

Direct Coombs Test

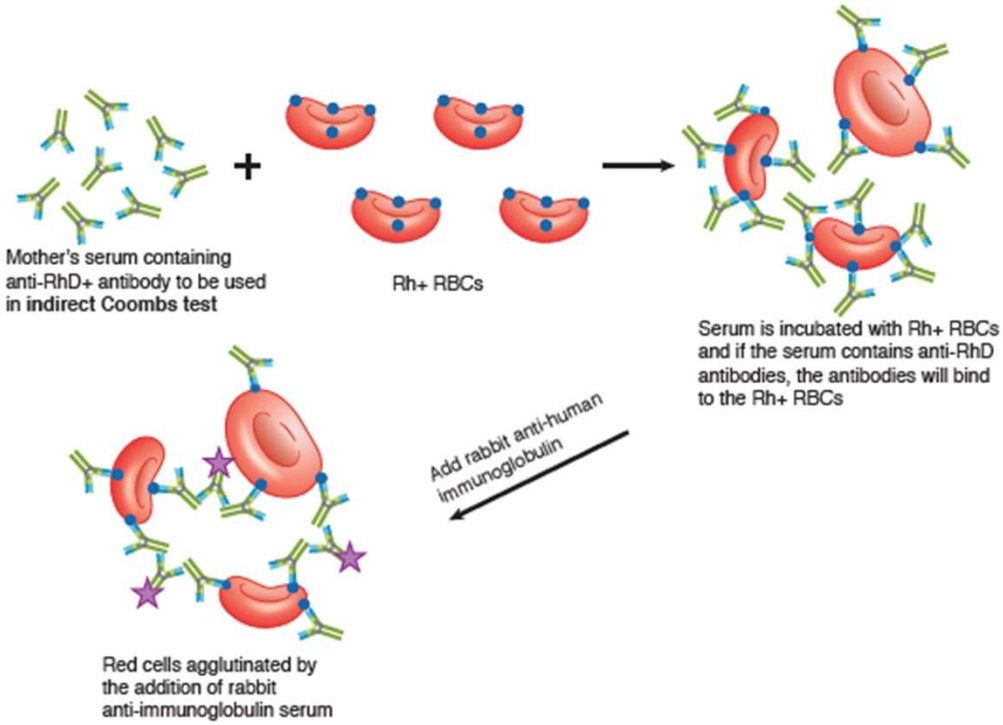


IMMUNODIAGNOSIS

The **indirect Coombs test** is designed to identify Rh-negative mothers who are producing anti-Rh antibodies of the IgG isotype, which may be transferred across the placenta harming Rh-positive foetuses. The indirect Coombs is also used in the diagnosis of transfusion reactions.

IMMUNODIAGNOSIS

Indirect Coombs test



IMMUNODIAGNOSIS

ABO TESTING

ABO blood typing is a uniform fi st step in all tissue transplantation because ABO incompatibilities will cause hyperacute graft rejection in the host. The ABO blood group antigens are a group of glycoprotein molecules expressed on the surface of erythrocytes and endothelial cells. Natural isohemagglutinins (IgM antibodies that will agglutinate the glycoprotein molecules on the red blood cells of nonidentical individuals) are produced in response to similar molecules expressed on the intestinal normal fl ra. A person is protected by self-tolerance from producing antibodies that would agglutinate his own red blood cells, but will produce those agglutinins that will react with the red blood cells from other individuals.

IMMUNODIAGNOSIS

ABO Testing

		Blood Type A	Blood Type B	Blood Type O	Blood Type AB
Serum from: Contains:	A Anti-B Antibodies	N	A	N	A
Serum from: Contains:	B Anti-A Antibodies	A	N	N	A
Serum from: Contains:	O Anti-A and Anti-B Antibodies	A	A	N	A
Serum from: Contains:	AB No Antibodies to A or B	N	N	N	N

A: agglutination
N: no agglutination

IMMUNODIAGNOSIS

LABELED ANTIBODY SYSTEMS

Labeled antibody systems are utilized for the detection of antigens, which may be either self or foreign. These antigens can be visualized using a combination of specific antibody that is labeled or tagged with a compound used for its detection. Common tags include fluorescent compounds and enzymes. Each of the following discussed is an example of a labeled antibody system. Additionally, fluorescent antibody tests and ELISAs can be done using either direct or indirect tests as described previously.

IMMUNODIAGNOSIS

Fluorescent Antibody Tests

- **Direct fluorescent antibody test (DFA)**
- **Indirect fluorescent antibody test**

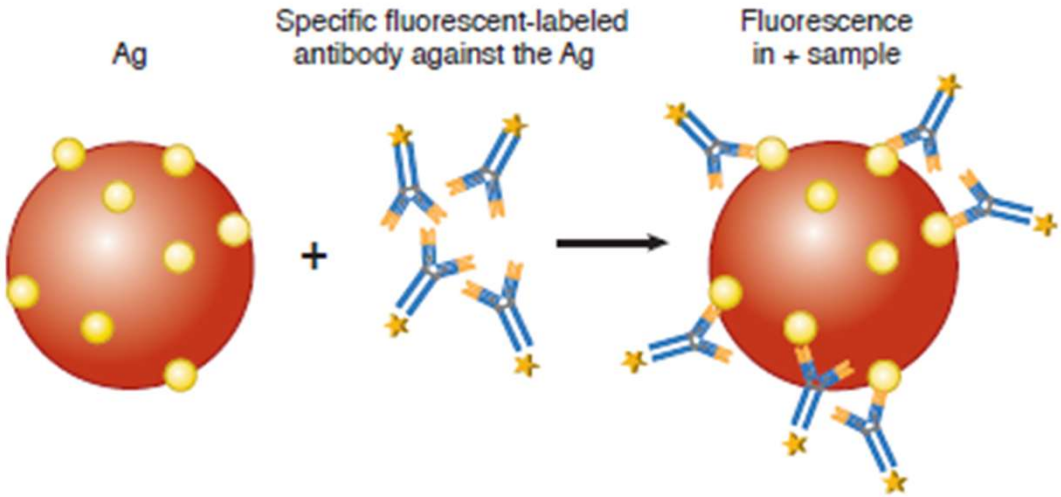
IMMUNODIAGNOSIS

Direct fluorescent antibody test (DFA) is used to detect and localize antigen in the patient. The tissue sample to be tested is treated with antibodies against that particular antigen that have been labeled with a fluorescent dye. If the antigen is present in the tissues, the fluorescent-labeled antibodies will bind, and their binding can be detected with a microscope. Variations of this test are used to diagnose respiratory syncytial virus, herpes simplex 1 and 2, rabies in animal tissues, and

- *Pneumocystis* infections.

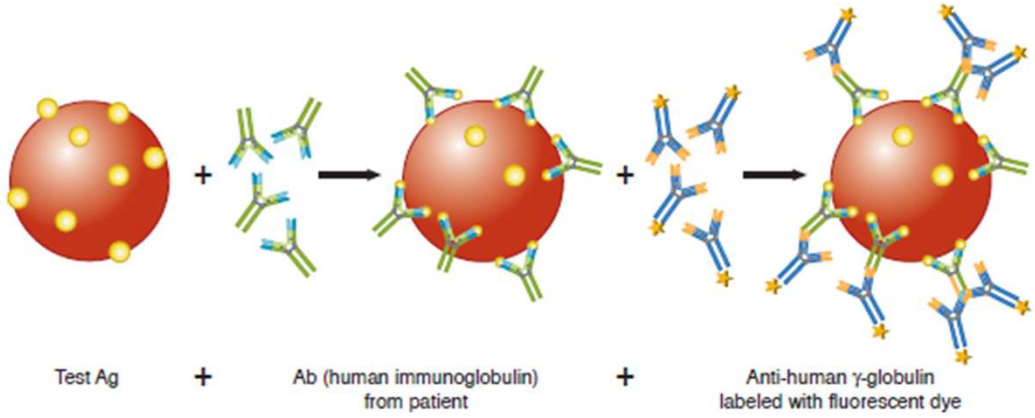
IMMUNODIAGNOSIS

DFA



IMMUNODIAGNOSIS

IFA



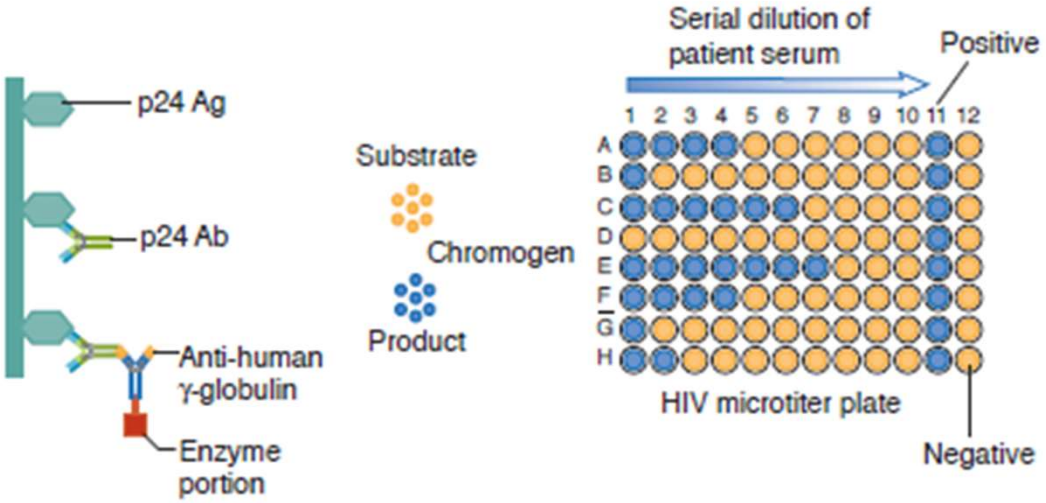
IMMUNODIAGNOSIS

Enzyme-linked Immunosorbent Assay (ELISA)

The ELISA is an extremely sensitive test (as little as 10–9 g of material can be detected). It can be used to detect the presence of hormones, drugs, antibiotics, serum proteins, infectious disease antigens, and Tumor markers. It does so by utilizing a chromogenic substrate that undergoes an enzyme-mediated colour change. In the screening test for HIV infection, the ELISA is used with the p24 capsid antigen coated onto microtiter plates. The serum from the patient is then added, followed by addition of an enzyme-labeled antihuman immunoglobulin. Finally, the chromogenic substrate is added, and the production of a colour change in thewell can be observed.

IMMUNODIAGNOSIS

ELISA



IMMUNODIAGNOSIS

Fluorescence Activated Cell Sorting (FACS)

Fluorescence activated cell sorting (FACS) is a procedure used to rapidly analyse cell types in a complex mixture. Th s is done by sorting the cells into different populations based on their binding to specific fluorescently labelled antibodies. By using antibodies against cell-surface markers conjugated to different fluorescent dyes, it is possible to analyse the relative numbers of cells present in a specific tissue location.

As cells pass through the apparatus in a single file, a computer-generated graph is produced, plotting the intensity and color of fluorescence of each cell along the axes. Each dot on the graph reflects the passage of a cell with a certain level and color of fluorescence, so the darkly dotted areas of the graph reflect the presence of many cells of similar attributes.

IMMUNODIAGNOSIS

FACS

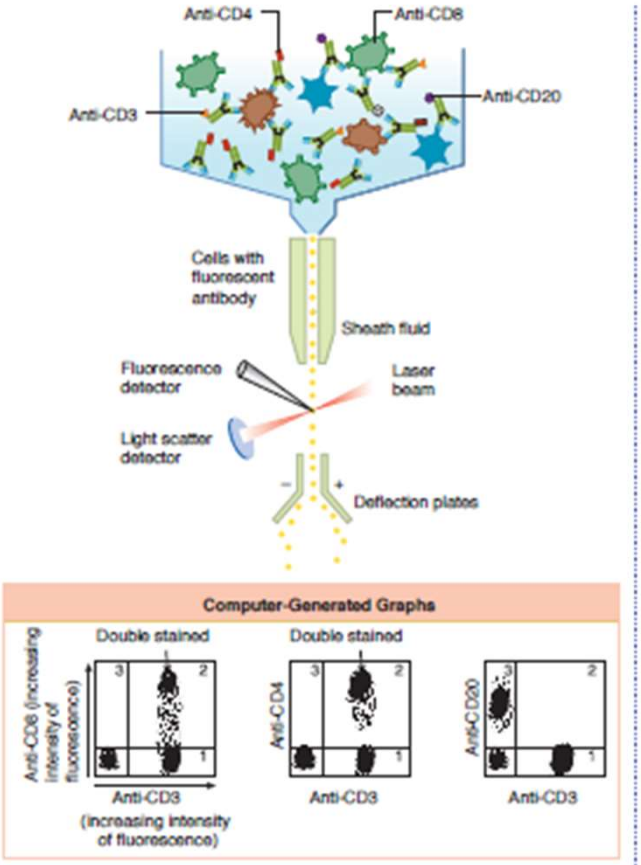


Figure I-9-16. Flow Cytometric Analysis

IMMUNODIAGNOSIS

Chapter Summary

• Antigen–antibody interactions can be visualized in vitro and serve as the basis of many medical diagnostic tests.

• Early in infection when antigen is in excess, only the pathogen's antigens can be detected in patient serum. As antibodies begin to be produced, complexes are formed that precipitate out of the circulation, and the patient enters the equivalence zone. Rising titres of antibody are measured as the patient progresses into the antibody excess zone, and convalescence.

• Agglutination tests are used to measure antibodies that can cause clumping of particles (RBCs and latex beads).

• The direct Coombs test is an agglutination test that detects infants at risk for developing erythroblastosis fetalis; the indirect Coombs test is used to diagnose the presence of antibody in mothers who are at risk of causing this condition in their children.

• The direct fluorescent antibody test is used to detect and localize antigen in patient tissues; the indirect fluorescent antibody test is used to detect antibody production in a patient.

• The enzyme-linked immunosorbent assay is an extremely sensitive test that can be modified to detect antigens or antibodies. The ELISA is used as a screening test for HIV infection.

• Flow cytometry is used to analyse and separate cell types out of complex mixtures.

IMMUNODIAGNOSIS