

# **CDC 4T051P**

## **Medical Laboratory Journeyman: Hematology, Immunology, and Blood Banking**

### **Volume 2. Immunology**



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This second volume of CDC 4T051P concerns Immunology. Unit 1 is an introduction to Immunology and discusses the immune response and antigen-antibody interaction. In Unit 2, Immunology laboratory environment, basic techniques, methodologies, and procedures are examined. This unit provides the foundation for all immunology testing. Unit 3, Immunology testing, reviews routine and viral test orders, their clinical significance, and laboratory identification.

A glossary of terms, abbreviations and acronyms used in this volume is included at the end of this book.

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In this volume, the subject matter is divided into self-contained units. A unit menu begins each unit, identifying the lesson headings and numbers. After reading the unit menu page and unit introduction, study the section, answer the self-test questions, and compare your answers with those given at the end of the unit. Then do the unit review exercises.

	<i>Page</i>
<b>Unit 1. Immunology Introduction .....</b>	<b>1-1</b>
1-1. The Immune Response .....	1-1
1-2. Antigen-Antibody Interaction.....	1-14
<b>Unit 2. Immunology Procedures.....</b>	<b>2-1</b>
<b>Unit 3. Immunology Testing.....</b>	<b>3-1</b>
3-1. Routine Testing .....	3-1
3-2. Viral testing.....	3-18
<i>Glossary.....</i>	<i>G-1</i>
<i>Bibliography .....</i>	<i>B-1</i>



# Unit 1. Immunology Introduction

<b>1–1. The Immune Response .....</b>	<b>1–1</b>
201. Immunity .....	1–1
202. Immunogens and antigens .....	1–5
203. Immunoglobulins and antibodies .....	1–8
<b>1–2. Antigen-Antibody Interaction.....</b>	<b>1–14</b>
204. Antibody production.....	1–14
205. The antigen-antibody complex .....	1–20

**T**HE IMMUNE phenomenon was first recognized when it was noticed that individuals who survived smallpox did not become reinfected. This discovery prompted the Chinese in 1500 A.D., to begin the risky practice of inhaling the crusts of smallpox lesions to prevent future infections. Western Europe saw its initiation into the field of immunology when Lady Montagu, the wife of the British ambassador to Turkey, introduced “*variolation*.” This procedure, involving the injection of smallpox crusts or blister fluid, became very prevalent in Europe and was successful in most cases. Its primary drawback was that the virus injected was infective and, therefore, of danger to the local community. An English physician named Edward Jenner published the first definitive work on safe vaccination in 1798. In this study, it was noted that a young boy, who was vaccinated with pus from an individual suffering from cowpox and later injected with smallpox, did *not* contract the disease. Dorland’s defines vaccination as “the introduction of a vaccine into the body for the purpose of inducing immunity.” It is derived from the Latin word *vacca*, or “cow” and was originally coined for the injection of smallpox vaccines. The meaning of vaccination now includes any vaccine injected to produce an immune response. Another term you are probably familiar with is immunization. It also means “the induction of immunity” but not necessarily from vaccines.

## 1–1. The Immune Response

The study of the immune response is *immunology*, defined as that body of knowledge concerned with the biological, chemical, and physical factors that contribute to the body’s resistance to immunogenic agents. Immunology is a biomedical science branch concerned with the body’s response to antigenic challenge or how it distinguishes between “self” and “non-self.” Many of the contributors to microbiology, found in 4T051B, are also recognized for their contributions to immunology. The study of microorganisms is incomplete without the study of the body’s response to such non-self or foreign invaders. Consequently, immunology began as a branch of microbiology. However, today it encompasses the study of the structure and function of the immune system (basic immunology), immunization, organ transplantation, blood banking, immunopathology (clinical immunology), laboratory testing of cellular and humoral immune function, molecular immunology, immunogenetics, and the use of antigen-antibody reactions in other laboratory tests (serology and immunochemistry).

### 201. Immunity

The three major functions of the immune response are (1) specificity, (2) distinguishing between self and non-self, and (3) memory of past invaders. As previously stated, the essence of immunology is understanding the importance of the body recognizing self from non-self for survival. Immunity is defined as the state of being immune or protected from various infectious diseases. Immunity includes *innate immunity*, *adaptive immunity*, and *adoptive immunity*. Let’s begin the study by taking a closer look at the body’s ability to fight foreign invaders through innate immunity.

**Innate immunity**

Innate immunity is a nonspecific immunity possessed by a certain animal species due to some special property of that species without the production of an antibody. It is also called *natural resistance*.

**Some illustrations**

Tuberculosis is common in humans, cattle, pigs, and various fowl, but it is very uncommon in sheep, cats, dogs, and horses. Such cold-blooded animals as snakes and turtles are *not* susceptible to gonorrhea, mumps, typhoid fever, or measles. (As a passing thought, can you imagine what a snake with mumps would look like?) Innate immunity is present from birth and the response is immediate.

**Defense mechanisms**

Innate immunity is the first line of defense against challenging organisms or foreign invaders. It provides unfavorable environments for the invaders. The overlapping defense mechanisms of innate immunity can be broken down into those of an *anatomical* nature and those, which are *chemical* in origin.

**Anatomical**

Some of the major players in anatomical defenses are shown in the following table.

Defense	Description
Skin	Intact skin is an effective physical barrier to most disease-causing organisms. Part of this defense mechanism is the acid pH of the skin surface, which inhibits some microscopic invaders.
Phagocytes	Kupffer cells of the liver and the microglial cells of the brain are examples of cells (from the mononuclear phagocytic system) that exist throughout the body which attack and destroy invading organisms.
Mucus	This body fluid, in conjunction with other mechanisms, such as the cough reflex and cilia of the respiratory tract, acts as a physical trap. Mucus may also contain phagocytes, enzymes, and antibodies which add effectively to the body's overall disease resistance.
Gastrointestinal tract	The simple act of swallowing moves potential pathogens to the stomach, where few can survive. Coughing, followed by swallowing, aids in the removal and destruction of respiratory pathogens. Many enzymes and other microbial inhibitors are also active in the salivary juices of the mouth.
Urinary tract	The flushing effect of the urinary tract helps to physically remove potential pathogens.
Eyes	The flushing action of tears, along with the presence of enzymes in the tears, provides one of the best examples of innate immunity. Acquired immunity is of minimal importance here. Even so, the eyes are perhaps the healthiest organs when one considers infectious disease.
Lymphatic system	This system of ducts running throughout the body has nodes at certain intervals. These nodes can physically filter microbes from lymphatic fluid. Also, the nodes are the sites of accumulation of white blood cells, including fixed tissue macrophages. These cells are then in an ideal position to phagocytize the offending microorganisms.



### Chemical

The following chemical defense mechanisms (excluding antibodies) influence the degree of immunity also.

Defense	Description
Lysozyme	This enzyme is present in many body fluids, such as mucus, tears, and saliva. It primarily attacks the cell walls of gram-positive bacteria
Interferon	This protein is produced by the body in response to a viral infection or after the injection of killed viral agents. It differs from antibodies in that interferon may provide protection against challenge by another, completely unrelated virus, while antibodies normally act only against the specific injected virus. Interferon can block the replication of viruses in other cells.
Properdin	This high molecular weight protein is effective against certain viruses and, also, against some gram-positive bacteria and even a few gram-negative ones. Properdin might be considered to be an antibody-like substance, since its action requires the presence of complement and magnesium ions, both of which are active in certain antibody reactions. In fact properdin has been called a "natural antibody," but it lacks the specificity of an antibody and attacks many different organisms.
Inflammation	The tissue invasion by certain microorganisms causes inflammation, bringing several defense mechanisms into play. Inflammation appears to represent an orderly sequence of coordinated events designed to protect the host from a foreign invader, minimizing damage to the host tissue. In addition to killing off the adversary, the inflammatory process is intended to eliminate debris and repair damaged tissues. There are 4 stages of a localized inflammatory reaction; (1) increased vascular permeability, (2) emigration of neutrophils, (3) emigration of mononuclear cells, and (4) cellular proliferation and repair. Additionally, there is usually a rise in temperature (fever), either in the immediate area or in the entire body, thus rendering a less favorable environment for most pathogens. All of these changes during inflammation combine to resist further reproduction and dissemination of the pathogen.
Complement	Complement refers to the group of proteins that mediate a cascade of enzymatic reactions. The <i>complement cascade</i> can be triggered by an antigen-antibody complex or by molecular features found on the surface of some microorganisms. The consequences of complement activation include production of inflammatory mediators, cytolysis or hemolysis of erythrocytes, and enhanced phagocytosis of the foreign invader. Mediators are cells that participate in an immunological reaction by releasing biochemical substances.
Acute-phase proteins	During serious infections the body produces a group of serum proteins known as acute-phase proteins. Some of the proteins have antimicrobial effects. For example: C-reactive protein binds to the so-called C-protein on the surface of pneumococci. This promotes their destruction by the complement cascade.
Opsonins	There are many different types of particles (for example; species of encapsulated bacteria) that can't be phagocytized directly. But, if coated with proteins produced by the host these particles can be phagocytized. Proteins that have the ability to enhance phagocytosis are known as opsonins. Immunoglobulins and complement derivatives are the most important.

**Adaptive immunity**

Adaptive immunity is an acquired or learned type of immunity. The term *acquired* denotes immunity, which is obtained after birth and is neither inherited nor innate. It is a delayed response to a specific stimulating challenge and is quite involved. Adaptive immunity can be divided into two forms: *humoral-mediated immunity* and *cell-mediated immunity* (discussed in greater detail in the next lesson). It can also be two different types: *active* and *passive* immunity.

***Humoral-mediated***

Humoral-mediated immunity is when the body recognizes a foreign substance and then produces antibodies against that specific substance. The primary purpose of this form of immunity is a defense against bacterial infections.

***Cell-mediated***

Cell-mediated immunity consists of T lymphocytes that regulate antibody reactions by helping or suppressing the activation of B lymphocytes. They also, recognize abnormal host cells, transplanted cells, and foreign organisms; and initiate a cellular response through the release of lymphokines or other chemicals. The primary purpose of this form of immunity is a defense against viral and fungal infections, intracellular organisms, tumor antigens, and graft or transplant rejection.

***Active***

Active immunity is when the host cells produce immune products to generate protection. Active immunity is either naturally or artificially acquired.

***Naturally acquired active***

To acquire active immunity by natural means involves contracting a disease and thereby producing an antibody to that disease. However, the fact that a person has recovered from an infectious disease and received a “naturally acquired active immunization” does not guarantee resistance to another attack by the same microorganism. For example, influenza and gonorrhea result in very short-lived immunity, and repeated infections are common. On the other hand, a single infection of the measles or chicken pox usually confers life-long immunity to reinfection. There are other examples of these extremes of immunity and, also, many that fall between the extremes.

***Artificially acquired active***

Whereas an individual may gain life-long immunity to smallpox through surviving the disease, vaccinations will also produce this response artificially, without the associated life-threatening disease process. For many diseases, vaccination has proven to be an effective method of providing artificially acquired active immunity. This immunity, however, is not absolute, and the relative efficiency of each vaccine can be estimated by the frequency that reimmunization is required. In endemic areas, cholera and plague shots are given every 6 months; whereas yellow fever shots are given every 10 years.

***Passive***

Passive immunity is from *preformed* (prepared in advance) immune products that are administered to provide protection. This immunity can also be acquired naturally or artificially.

***Naturally acquired passive***

This type of immunity is important in the survival of newborn infants. The infant passively acquires antibodies from its mother. The antibodies may pass from the immune mother to the fetus across the placental barrier or through the colostrum of breast milk. This is relatively short-lived immunity, seldom lasting more than six months.

*Artificially acquired passive*

Antibodies that have been produced in another individual or animal and then administered by injection provide this type of immunity. This method has been used extensively in the treatment of diphtheria, tetanus (tetanus toxoid), and hepatitis (gamma globulin). The immunity gained by passive immunization is usually only temporary, but it is extremely efficient.

*Review*

The different types of immunity can be confusing and hard to distinguish. See the table below for a quick review.

Type	Method of Acquisition	Antibody production by host	Time of immune response
Naturally acquired active	Contacting disease or Infection	Yes	Long term
Artificially acquired active	Vaccination	Yes	Long term
Naturally acquired passive	Transfer from mother to infant	No	Short term
Artificially acquired passive	Administration of serum or plasma	No	Short term

**Adoptive immunity**

A bone marrow transplant is an example of adoptive immunity. A bone marrow transplant essentially takes immunocompetent cells from a healthy person and transfuses them into a diseased patient. The immunocompetent cells are used to “reconstruct” the immune system of an immunoincompetent individual.

**202. Immunogens and antigens**

There are many natural and synthetic substances that can evoke an immune response. This response can be humoral, cellular, or both. The classic term to define these substances is *antigen*, however, the more correct term is *immunogen*.

**Immunogens**

An immunogen is a substance that will elicit an immune response when introduced into the tissues of a normal, immunocompetent individual or host. Normally, immunogens are foreign to the host and have a molecular weight of greater than 10,000 daltons. Nonetheless, only a small portion of the molecule can combine with an antibody.

***Antigenic determinant or epitope***

The specific set of chemical properties that is acknowledged by a given antibody or T cell receptor is called an *antigenic determinant* or *epitope*. The degree to which a substance may produce an immune response is referred to as its *immunogenicity* or *antigenicity* (for antigens).

***Factors of immunogenicity and antigenicity***

What makes a substance immunogenic and to what degree? The exact mechanisms are unknown; however, there are several factors that contribute to its immunogenicity. In order for a substance to elicit an immune response it must not only be foreign but its molecular size, chemical composition and complexity, genetic composition of the host, and the method of administration is also important.

***Molecular size***

Extremely small molecules (amino acids or monosaccharides) less than 1,000 daltons are rarely immunogenic. Those molecules below 10,000 daltons are weakly immunogenic, but, the most potent immunogens are proteins over 100,000 daltons.

*Chemical composition*

Large, macromolecular proteins and complex carbohydrates provide the most potent immunogens. Polysaccharides and short polypeptides usually produce weak responses. Pure lipids and nucleic acids have not been shown to produce an immunogenic response. However, anti-DNA antibodies found in patients with systemic lupus erythematosus suggest that they can be antigenic but not immunogenic.

*Chemical complexity*

Homopolymers of a single amino acid and monosaccharides are poor immunogens regardless of their size. If the molecule has 2 or 3 amino acids it is usually more active; the more complex the molecule is, the more potent the immunogen. (Example: aromatic amino acids such as tyrosine.)

*Genetic composition of the host*

The genetic composition of the host plays an important role in immunogenicity. A specific polysaccharide will produce an immune response when injected into humans, but not when injected into guinea pigs. The ability to respond to a certain immunogen is genetically predetermined. Crossbreeding studies with guinea pigs prove this true. Also, in humans, research in the area of the major histocompatibility complex (MHC) continues to suggest that certain individuals with specific human leukocyte antigen (HLA) antigens have various responses to particular immunogens.

*Method of administration*

It is still unclear why dosage, route, and timing of immunogen administration affect the immune response. It is known that if a dose of an immunogen is too small then a response may not occur at all. If the dose is too large it may cause a high-zone tolerance which is also a state of unresponsiveness. The proper dose for some immunogens is very important. The immunogenicity of a substance may depend on whether it is injected intramuscularly or intravenously or taken orally. Most immunogens react better if injected, but, an exception is the polio vaccine. It elicits a better response when taken orally.

**Haptens**

There are many compounds that can combine with components of the immune system, but they don't produce an immune response. Some examples are low molecular weight compounds such as antibiotics and other drugs. Haptens are low molecular weight substances that can combine with antibodies but they don't evoke an immune response. But, if a hapten combines with an immunogenic carrier molecule, then the hapten also becomes immunogenic. Antibodies are produced to the hapten, the carrier, and the hapten-carrier complex. Because antibodies are produced to all three substances, it was concluded that antibodies react very specifically and can recognize three-dimensional structures.

**Antigens**

Antigen refers to the ability of a substance to combine with an antibody or T cell receptor. The antigen generates antibodies or lymphocytes that react specifically with that antigen. All immunogens are antigens, but not all antigens are immunogens. For the rest of our study we will use the term antigen because we are mostly interested in antibody production in serological testing.

*Epitope*

As stated earlier the part of the antigen that binds to the antibody or T cell receptor is called the epitope. An antigen may have one epitope, many epitopes of the same specificity, or many epitopes with different specificities. For a schematic example, see figure 1-1.



One epitope



Many epitopes of the same specificity.



Many epitopes of different specificities.

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Figure 1-1. Schematic of possible antigen structures.

### **Major classes**

The major classes of antigens are carbohydrates and proteins. The cellular membrane of mammalian cells are composed of proteins, phospholipids, cholesterol, and traces of polysaccharides (glycoproteins or glycolipids). Do you recall from 4T051C, volume 1, *Hematology* what the cellular membrane of erythrocytes and leukocytes are made of? How about 4T051B, volume 1, *Bacteriology Introduction* on the composition of a bacterial cell wall? The statement above should sound familiar. (If these chemical components are antigenic, it is reasonable and understandable why blood cells and bacteria are antigenic.) The cellular antigens most important to the laboratory technician are the blood group antigens and the major histocompatibility complex. The proteins and lipids found on these cells become immunogenic when transfused into a different person of the same species. Microbial antigens are also important because through their production of antibodies they allow the study of immunologic manifestations of infectious diseases.

### **Blood group antigens**

Blood group antigens will be discussed in detail in the next volume *Blood Banking*. Keep in mind the principles of antigen-antibody reactions for the next volume.

### **Major histocompatibility complex**

The major histocompatibility complex (MHC) is often referred to as the HLA (human leukocyte antigen) system because its gene products were originally identified on white blood cells. The MHC plays an important role in immunological studies. The MHC has three classes of antigens: Class I, Class II, and Class III. Class I antigens are a single transmembrane glycoprotein chain with the majority of the amino acids on the exterior of the cellular membrane. They are found on almost all nucleated cells and are recognized in graft rejection. Class II antigens are two glycoprotein transmembrane chains also with the majority of the amino acids on the exterior of the cellular membrane. They are found on immunocompetent B cells and macrophages and are important for antigen presentation and interactions between immunocompetent cells. Class III antigens are serum complement components that activate complement factors. The application of the MHC is significant in organ transplantation, bone marrow transplantation, disease association, parental or family studies, forensic medicine, and in regulating of the immune response during antigen presentation.

### Autoantigens

When the immune system recognizes a foreign invader as non-self and seeks to destroy it, the immune system is wonderful. But, when this powerful system goes haywire and starts to attack ones self, then something is wrong. The failure of the immune system to recognize self-antigens results in the production of autoantibodies. These autoantibodies can attack hormones, cells, or organ tissues and, in turn, harm the host.

## 203. Immunoglobulins and antibodies

Antibodies are specific glycoproteins called *immunoglobulins* produced by B lymphocytes and plasma cells as the result of stimulation by an antigen. All antibodies are capable of chemical union both *in vivo* and *in vitro* with the particular antigen responsible for their production (homologous antigen).

### General characteristics of immunoglobulins

Immunoglobulins have two important characteristics: (1) *specificity* and (2) *biological activity*. Specificity limits an antibody to combine or attach to an identical antigenic determinate or epitope. However, because there are many different antigen structures, the immunoglobulins must also be diverse. The immune system is able to produce a variety of immunoglobulin molecules for each antigen. Their biological activities are numerous. The following is a general list of these activities. Keep in mind that not all immunoglobulin or antibody isotypes can perform all of these activities.

List of Biological Activities
Neutralization of toxins and viral activity by binding, sequestering, and inactivating toxic proteins or by blocking receptors on a viral particle so that the virus can't adhere to the host cells.
Immobilization of microorganisms.
Agglutination of microorganisms or antigenic particles.
Binding with soluble antigens leading to the formation of precipitates which are destroyed by phagocytes.
Activating serum complement to facilitate the lysis of microorganisms.
Activating phagocytic cells.
Crossing the placental barrier.

### Composition of immunoglobulins

Immunoglobulins are composed of 82 to 96% polypeptide and 4 to 18% carbohydrate by weight. They have two functions as related to their characteristics: (1) they bind to specific antigens and (2) initiate a variety of secondary immune responses.

### Immunoglobulin structure

All immunoglobulins consist of 2 different types of polypeptides. The larger type is called a heavy (H) chain and the smaller is known as a light (L) chain. The heavy chain is roughly twice as large as the smaller chain. Each immunoglobulin molecule contains an equal number of heavy and light chains (2 identical chains of each). These chains are held together by noncovalent forces and also by covalent interchain disulfide bonds. Both chains are composed of a number of folded globular *domains*—represented by the half circles in figure 1–2. Each domain is composed of 100 to 110 amino acids and is connected by a single intrachain disulfide bond. Light chains contain 2 of these domains and heavy chains contain 4 or 5 domains each. The *variable domain* is located in the N-terminal of these chains. It is this area of the chain that determines the immunoglobulins specificity and, therefore, its ability to combine with specific antigens. The variable domain is designated with a “V.” For example;  $V_H$  designates the variable domain on the heavy chain and  $V_L$  for the variable domain on the light chain. The *constant domain* amino acids are less variable. The constant domains in the heavy chain significantly affect the immunoglobulins biological activities. The constant domain is designated with a “C.” For example;  $C_H$  designates the constant domain(s) on the heavy chain and

$C_L$  for the constant domain on the light chain. The heavy chain constant domains are numbered sequentially ( $C_{H1}$ ,  $C_{H2}$ ,  $C_{H3}$ ,  $C_{H4}$ , and  $C_{H5}$ ) beginning with the closest to the  $V_H$ . Every immunoglobulin is composed of one or more of these basic 4-chain units.

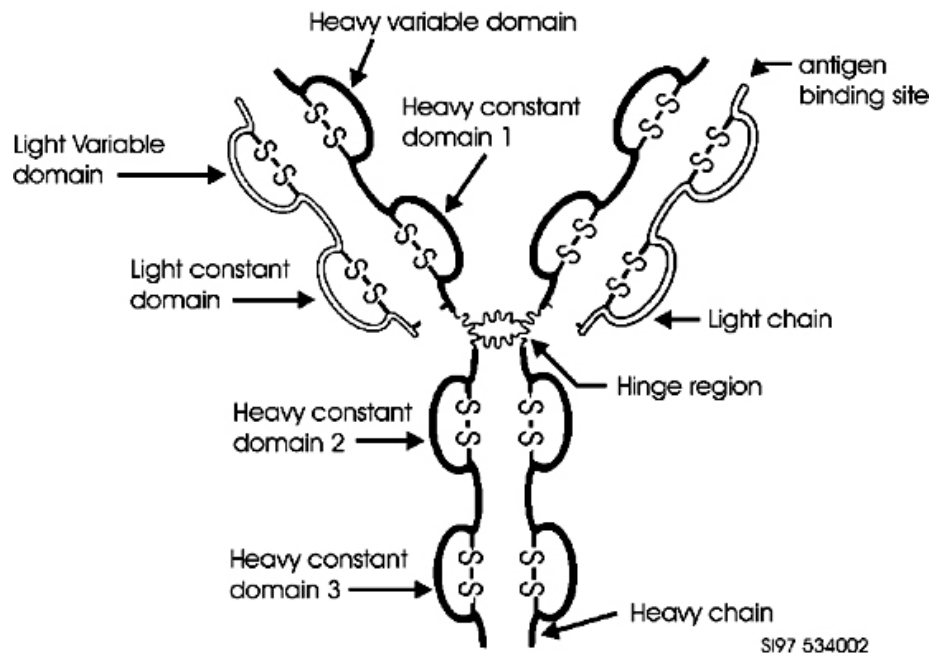


Figure 1-2. Schematic of the basic 4-chain immunoglobulin molecule.

### Immunoglobulin fragments

Immunoglobulins can be split into 3 similar fragments: two identical monovalent antigen-binding fragments (Fab fragments) and one crystallizable fragment (Fc fragment). The Fc fragment can't bind to an antigen but it is recognized by the Fc receptor on different cells.

### Immunoglobulin classes

Specific amino acid sequences characterize chains of the different immunoglobulin *classes* or *isotypes*. There are two types of the light chains: kappa ( $\kappa$ ) and lambda ( $\lambda$ ). In any one immunoglobulin molecule the light chains are always either  $\kappa$  or  $\lambda$ , never one of each. At this time there are no known functional differences between these light chains. The major heavy chain classes are alpha ( $\alpha$ ), delta ( $\delta$ ), epsilon ( $\epsilon$ ), gamma ( $\gamma$ ), and mu ( $\mu$ ). The heavy chains account for the major, identifiable, physical differences seen in the various immunoglobulins. All the heavy chains in a given immunoglobulin molecule are also identical. The heavy chains are designated IgG ( $\gamma$ ), IgM ( $\mu$ ), IgA ( $\alpha$ ), IgD ( $\delta$ ), and IgE ( $\epsilon$ ). Either  $\kappa$  or  $\lambda$  light chains can be mixed with any of the different heavy chains.

### IgG

IgG makes up the largest class of immunoglobulins in the peripheral blood and is the only antibody that can pass through the placental barrier from the maternal circulation to the fetal circulation. It is also found in lymph, cerebrospinal, and peritoneal fluids. IgG can be divided into four subclasses: IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, and IgG<sub>4</sub>. The four subclasses are based on differences in their respective amino acid sequence in the heavy chain. IgG<sub>3</sub> binds well with complement while IgG<sub>1</sub> and IgG<sub>2</sub> bind poorly, and IgG<sub>4</sub> binds complement only under certain conditions. The actual biological significance of these subclasses is still under scrutiny by the scientific community. Figure 1-3 shows a three-dimensional model of an IgG<sub>1</sub>. Be aware that all immunoglobulins are actually three-dimensional in the body.

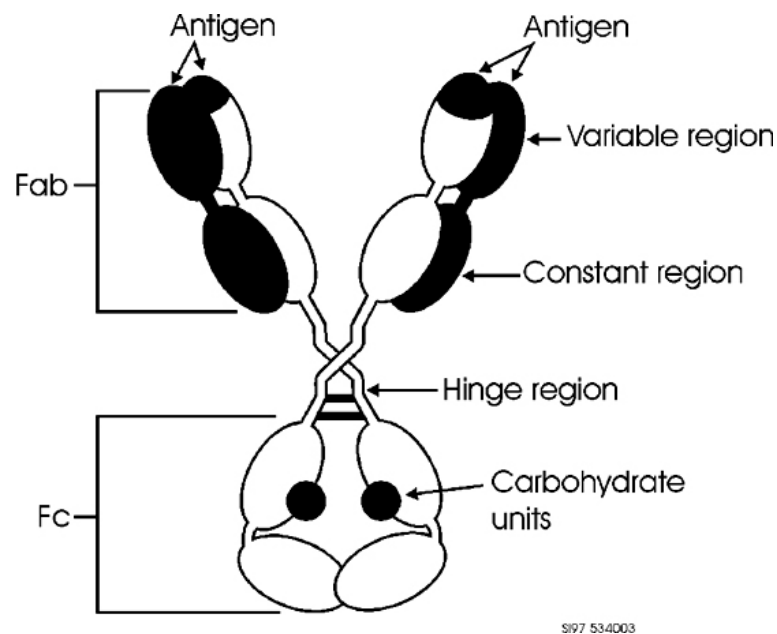


Figure 1-3. A three-dimensional model of IgG<sub>1</sub> structure.

### *IgM*

This class of immunoglobulins is the first produced by the developing fetal system and is the primary class of antibody seen in the beginning stages of early antibody response. IgM, a 19S globulin, is characterized by mu ( $\mu$ ) heavy chains. It is a pentamer macroglobulin that is composed of 5 basic 4-chain units with 5 globular domains (one  $V_H$  and four  $C_H$ ) on the heavy chains. It is also polyvalent which means it can bind to 10 identical antigen-binding sites. It also contains an additional polypeptide called a joining (J) chain as shown in figure 1-4.

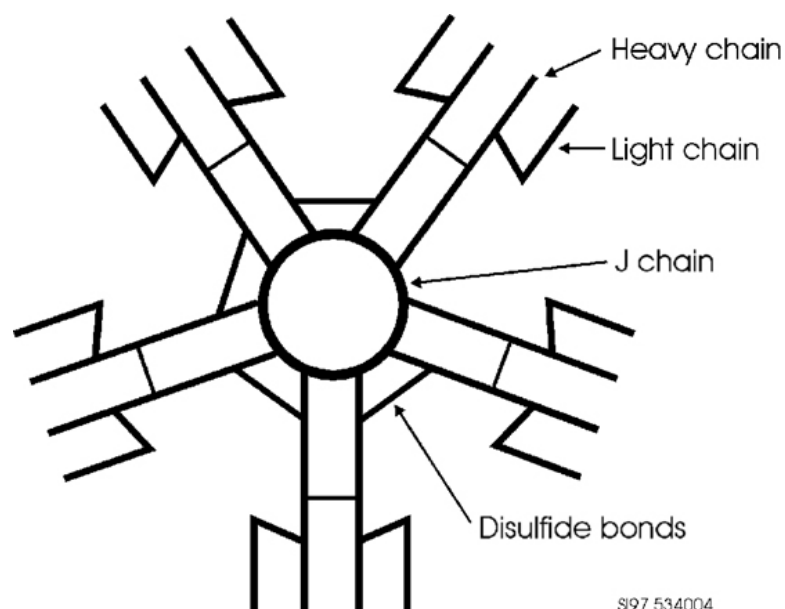


Figure 1-4. A schematic of an IgM immunoglobulin.



### ***IgA***

IgA immunoglobulins are characterized by alpha heavy chains and their 7S sedimentation coefficient. These antibodies are found in bodily secretions: saliva, tears, and fluids of the respiratory and gastrointestinal tracts. It is also the major immunoglobulin in the colostrum. If IgA is present in mucous secretions it exists as 2 basic 4-chain units as shown in figure 1-5. It is linked by the same J chain found in IgM molecules. It also has an attached protein; the secretory component or S piece. If the IgA is present in serum, it is predominantly a single basic 4-chain unit molecule without the secretory component. There are two antigenically distinct subclasses in humans, IgA<sub>1</sub> and IgA<sub>2</sub>.

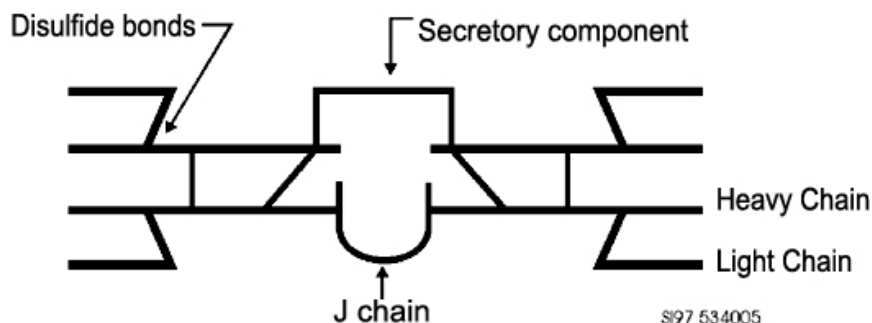


Figure 1-5. A schematic of an IgA immunoglobulin.

### ***IgD***

IgD is characterized by delta heavy chains and its very low levels in serum. It consists of 1 basic 4-chain unit. Primary concentrations of this class may be found on the surface of B lymphocytes. Its exact function is unknown at this time.

### ***IgE***

Practically all of the body's IgE can be found in basophilic granulocytes and their tissue equivalent mast cells. It is the combination of this immunoglobulin with specific antigens which triggers the basophil to release histamine and vasoconstrictor substances. This reaction in the tissues can lead to various clinical manifestations, such as a skin rash, edema, and respiratory constriction. IgE is an 8S immunoglobulin and is characterized by epsilon ( $\epsilon$ ) heavy chains. It is a basic 4-chain unit molecule and, like the IgM, it also consists of 5 heavy chain domains. This extra domain attaches with unusually high affinity to specific receptors on basophils and mast cells, where it may remain for weeks or months. IgE may play a role in the protection against parasites.

### ***Review***

The table below is a review of the different characteristics of the immunoglobulins.

	<b>IgG</b>	<b>IgM</b>	<b>IgA</b>	<b>IgD</b>	<b>IgE</b>
<b>Molecular weight (approximate)</b>	150,000	900,000	160,000 or 400,000	180,000	190,000
<b>Carbohydrate content</b>	2 to 3%	12 to 14%	7 to 11%	12 to 14%	12 to 14%
<b>Sedimentation coefficient* (Svedberg units)</b>	6 to 7S	19S	7S	7-8S	8S
<b>Electrophoretic mobility (average)</b>	$\gamma$	Fast $\gamma$ to $\beta$	Fast $\gamma$ to $\beta$	Fast $\gamma$	Fast $\gamma$
<b>Half-life days</b>	23	5	6	3	2

	<b>IgG</b>	<b>IgM</b>	<b>IgA</b>	<b>IgD</b>	<b>IgE</b>
<b>Cross placenta</b>	Yes	No	No	No	No
<b>Normal serum level (mg/dl)</b>	800 to 1,600	50 to 200	150 to 400	1.5 to 4.0	0.002 to 0.05
<b>Heavy chain class</b>	$\gamma$	$\mu$	$\alpha$	$\delta$	$\epsilon$
<b>Heavy chain subclasses</b>	$\gamma 1, \gamma 2, \gamma 3, \gamma 4$		$\alpha 1, \alpha 2$		
<b>Light chain type</b>	$\kappa$ and $\lambda$	$\kappa$ and $\lambda$	$\kappa$ and $\lambda$	$\kappa$ and $\lambda$	$\kappa$ and $\lambda$
<b>Antiviral activity</b>	+	+	+++	?	?
<b>Bacterial lysis</b>	+	+++	+	?	?
<b>Complement fixation</b>	+	++++	0	0	0
<b>General comments</b>	Accounts for $\cong^{**}$ 75% of total serum immunoglobulin	Accounts for $\cong^{**}$ 10%	Accounts for $\cong^{**}$ 10 to 15%	Only found in trace amounts in serum because it is found on the surface of B cells	Accounts for $\cong^{**}$ 0.004%
<p>*Through ultracentrifugation, immunoglobulins have been labeled according to their sedimentation properties in Svedberg (S) units. (There is, of course, a relationship between molecular weight and sedimentation coefficient.)</p> <p>**Approximately (<math>\cong</math>)</p> <p>?Antiviral activity and bacterial lysis unknown at this time.</p>					

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 201. Immunity

1. What is the definition of innate immunity?
2. What are the major players in the anatomic defense?
3. What are the chemical defense mechanisms?
4. What is the definition of adaptive immunity and what are its two forms?
5. What is humoral-mediated immunity?
6. What is cell-mediated immunity?

7. What is the difference between naturally acquired and artificial acquired active immunity?
8. What is the difference between naturally acquired and artificially acquired passive immunity?

**202. Immunogens and antigens**

1. What is an immunogen?
2. What is an epitope?
3. What is the definition of immunogenicity or antigenicity?
4. What are the factors that contribute to a substances immunogenicity?
5. What are haptens?
6. What is the definition of an antigen?
7. What are the major classes of antigens?
8. What are the cellular antigens most important to the laboratory technician?
9. To what areas of medicine is the application of the MHC significant?

**203. Immunoglobulins and antibodies**

1. What are the two important characteristics of immunoglobulins?
2. What are the biological activities of immunoglobulins?
3. What are the two functions as related to their characteristics?
4. What are the two types of polypeptides in immunoglobulins and their description?

5. What are the 3 fragments of immunoglobulins?
6. How does the Fc fragment work?
7. What are the two types of light chains and the major classes of the heavy chain?
8. What are the heavy chain designations?
9. What is the largest class of immunoglobulins and where are they found?
10. What is the first immunoglobulin produced and what is it composed of?
11. Where is IgA found?

## **1-2. Antigen-Antibody Interaction**

We have covered the origins and characteristics of antigens and antibodies, including a detailed discussion on immunoglobulins. Now we will elaborate on antibody production and principles of antigen-antibody interactions, which are specifically related to the field of immunology and serology procedures. A brief study of complement will also follow.

### **204. Antibody production**

Since the 1960's, knowledge in the area of immune response and antibody production has grown by quantum leaps. The growth was largely spurred by the need for tissue transplants and AIDS research. When an antigen enters a normal immunocompetent host, a chain of events is set in motion that will end in the production of an antibody. The antigen may be a living organism, a killed organism, or in the case of tissue transplants, it may be tissue incompatible with the recipient, causing an antibody response.

#### **Cells involved in the immune response**

The cells primarily involved in the immune response are neutrophils, monocyte-macrophages, and lymphocytes. Neutrophils and monocyte-macrophages are phagocytic cells that act primarily by engulfing and digesting bacteria, cellular debris, and other particulate matter. Lymphocytes participate in both cellular and humoral responses. Although the cells are different; they work together, are dependent on each other, and are very important for good health.

#### ***Neutrophils***

During injury or infection, great numbers of neutrophils adhere to the blood vessel wall and then migrate to the affected area. At the site of injury or infection, there is a release of cellular chemical mediators, which produce swelling, redness, heat, and pain. These responses along with the increased presence of numerous neutrophils at the site are known as acute inflammation. The neutrophils immediately begin the process of engulfing any bacteria, cellular debris, or foreign particulate matter present at the site. Neutrophilic granules contain many different enzymes and other substances that

can kill and degrade bacteria or dissolve other particulate matter. A defense system based entirely on neutrophils is incomplete. The neutrophil response is limited to phagocytosis and intracellular or extracellular release of their granular contents. Neutrophils can't respond to certain types of toxins or viral particles and they don't possess "memory" capabilities.

### ***Monocyte-macrophages***

Monocytes are produced in the bone marrow, circulate in the peripheral blood, and migrate to the tissues where they become macrophages or histocytes. As stated in volume 1, *Hematology* they are part of the mononuclear phagocytic system. Once the monocyte migrates to a specific tissue or organ, it may undergo morphological changes due to the factors present in the new environment. Tissue macrophages live for 2 to 4 months and some are immobile while others are continuously roaming around. Certain stimuli activate the macrophage and increase its mobility, metabolic rate, and phagocytic activity. As phagocytic cells, they engulf particulate and soluble materials, subjecting them to the effects of numerous enzymes and other proteins. Macrophages secrete over 100 different products. Among the roles macrophages play in the immune response the most important is their ability to control the action of lymphocytes. They do this in two ways (1) by secreting potent immunoregulatory peptides [*tumor necrosis factor* (TNF) and *interleukin-1* (IL-1)] that control lymphocyte proliferation, differentiation, and effector function; and (2) acting as antigen-presenting cells. Some of the most important regulatory factors are in two diverse families of polypeptide hormones; *colony-stimulating factors* and *interleukins*. A brief discussion of colony-stimulating factors was presented in volume 1, *Hematology*. Interleukin is a generic term used for a group of unrelated protein factors produced by macrophages and T cells. Interleukins can also be *cytokines*. Cytokines are protein molecules that essentially transmit messages between cells. Cytokines that are produced by monocytes are *monokines* and those produced by lymphocytes are *lymphokines*. Antigen-presenting cells are responsible for processing and displaying foreign substances in a form that can be recognized by lymphocytes. Macrophages play a crucial role in initiating and coordinating nearly all types of immune responses.

### ***Lymphocytes***

Lymphocytes emerge from the primary lymphoid organs (thymus or bone marrow) in a resting state and are known as virgin cells. They migrate into various secondary or peripheral lymphoid organs (spleen, lymph nodes, or tonsils) where their encounter with foreign substances is maximized and they become activated. Lymphocyte proliferation takes place through two mechanisms: through lymphopoiesis and in the peripheral tissues once they become activated as part of an immune response. The latter mechanism gives rise to long-lived memory cells and also short-lived effector cells. (Effector cells are cells that actively carry out an immunologic attack.) Each cell carries out a specific function depending on the effector cell or lineage from which it arose, i.e., T cell or B cell.

#### ***T lymphocytes***

T cells arise from the bone marrow but migrate to the thymus, where they mature and acquire an array of surface antigens that reflects both maturational phase and functional capacity. T cells don't express immunoglobulins, but detect the presence of foreign substances by way of surface proteins called *T cell receptors*. T cells can only recognize a foreign protein if it is first broken down into small peptides and then displayed on the surface of a second host cell (antigen-presenting cell or APC). They must actually touch the surface of other cells to detect antigens as well as to produce most of their immunological effects. T cells express a variety of characteristic surface proteins, other than T cell receptors. Many of these proteins are referred to by the initials *CD* which stands for *cluster of differentiation*. Functionally distinct subpopulations of T cells include helper cells, suppressor cells, and cytotoxic T cells (effector cells). Helper cells promote the effector activities of both T and B cells, and stimulate macrophage activities. Suppressor cells inhibit T and B cell activities.

### *Cytotoxic T cells*

Cytotoxic T cells are a subpopulation of T cells that destroy foreign or altered cells by direct cell-to-cell contact without the presence of antibody. They can kill cells that have foreign macromolecules on their surface and they are extremely important against viral infections. Host cells that are infected by a virus can often be identified by the presence of viral peptides on their surface and killing these cells is essential to eradicate the disease.

### *Surface molecules on T cells*

The table below list some of the most important surface molecules on T cells.

<b>T cell marker</b>	<b>Major function or significance</b>
T cell receptor	Antigen binding
CD3 complex	Signal transduction from T cell receptor (lineage-specific marker)
CD2, CD5, CD7	Lineage-specific markers
CD4	Subset-specific marker (mainly on helper cells); interaction with Class II MHC proteins
CD8	Subset-specific marker (mainly on cytotoxic cells); interaction with Class I MHC proteins
IL-2 receptor, Class II MHC proteins, Transferrin receptor, CD25, CD29, CD54, CD69	Activation-specific markers
IL-1, IL-6, TNF $\alpha$ receptors	Other cytokine receptors
Fc receptors	Immunoglobulin binding
Lymphocyte functional antigen and intracellular adhesion molecule	Cell-cell adhesion molecules

### *B lymphocytes*

B lymphocytes have the capacity to synthesize the heavy and light chains described earlier and to assemble them into immunoglobulin molecules. B cells differentiate into memory B cells or plasma cells. Resting B cells (virgin or memory) can express tens of thousands of membrane immunoglobulins on their surfaces. The plasma cell actually secretes large amounts of immunoglobulins, but, oddly, the immunoglobulins are usually not present on its surface membrane. The plasma cell is oval or egg-shaped with an abundant cytoplasm and an eccentric round, “pinwheel” nuclei. The main function of the B cell is to secrete antibodies into the blood and other body fluids. This makes these environments unfriendly to foreign invaders. Additionally, B cells function as antigen-presenting cells for T cells and activated B cells that secrete lymphokines, which influence growth and functions of other cells.

### *Natural killer cells*

Natural killer cells are a group of lymphocytes that contain cytoplasmic granules and are capable of destroying virus-infected cells, neoplastic or allogenic cells. Natural killer cells are able to lyse virally infected cells and tumor cells without prior immunization. Unlike cytotoxic T cells, the cytolytic activity of natural killer (NK) cells is not major histocompatibility complex (MHC) restricted.

### **Production of antibodies**

A cell-mediated immune response involves T cells, cytotoxic T cells, and lymphokines. If a T cell destroys a target by direct contact, lymphokines recruit and activate other cells such as macrophages that in turn amplify the response. A humoral-mediated immune response involves the production of antibodies. For this lesson, the immune response and antibody production will be broken down into steps. However, these steps happen simultaneously to many different cells.

### ***Step 1***

Most immune responses must begin with the presentation of antigen through macrophages called APCs. Once the antigen enters the host it is bound to the APCs. Certain antigens remain on the surface and are

presented to specific lymphocytes but most are phagocytized. Some of the digested antigen particles are returned to the surface where they are more efficiently recognized by particular lymphocytes. As shown in figure 1-6, the APC captures the antigen (in this case a virus), digests it, and binds the viral peptide particles (epitopes) to the MHC class II molecule.

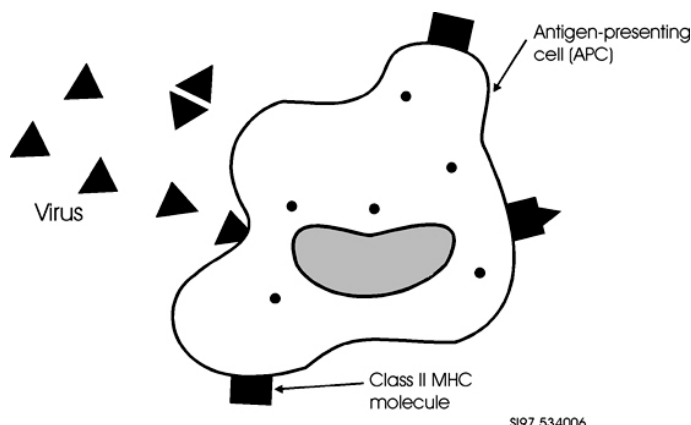


Figure 1-6. Step 1 in the sequence of an immune response.

### Step 2

Once the viral epitope is “presented,” the CD4 (helper) T cell receptor can detect the antigen that is bound to the MHC, but only if the T cell receptor protein can recognize and bind to that particular peptide-MHC complex, as shown in figure 1-7. The helper T cell then becomes activated through the binding and signals sent from the APC mediators (interleukins). Helper T cell activation is necessary for nearly all immune responses. As you can see the MHC class II-bearing APC, also, plays a pivotal role in controlling immune responses.

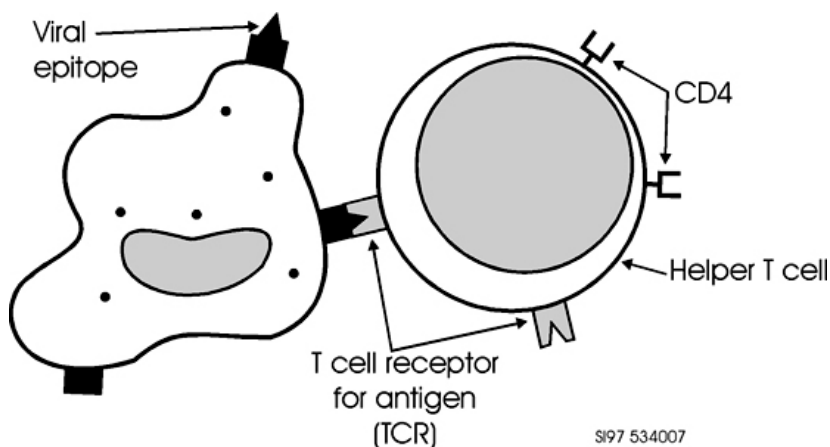


Figure 1-7. Step 2 in the sequence of an immune response - antigen processing and T cell activation.

### Step 3

The activated helper T cell begins to secrete the cytokine known as interleukin-2 (IL-2) and expresses specific high-affinity IL-2 receptors on its surface. IL-2 is important for the proliferation and differentiation of T cells into memory helper T cells or cytotoxic T cells. Helper T cells also produce and secrete other cytokines (for example; IL-4 and IL-6) that promote the growth, differentiation, and function of B cells, macrophages, and other cell types represented in figure 1-8.

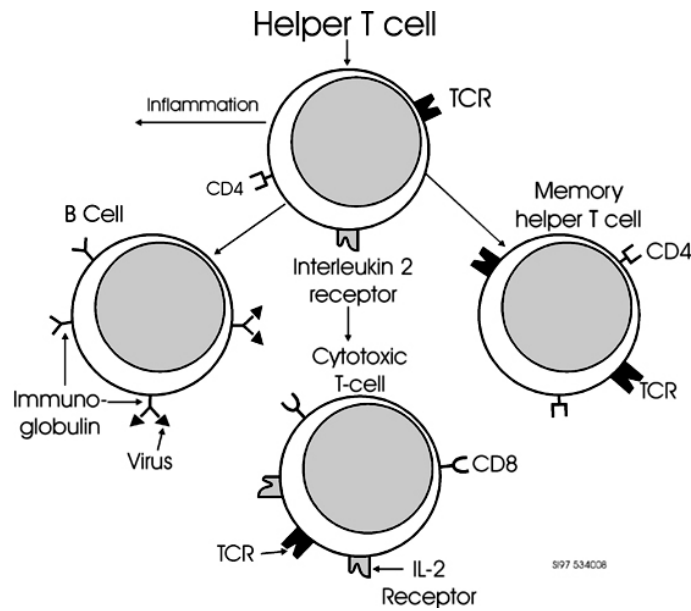


Figure 1-8. Step 3 in the sequence of an immune response - activation of memory helper T cells, cytotoxic T cells, and B cells.

### *Cytotoxic T cell activation*

Cytotoxic T cells can destroy virally infected or target cells without the involvement of an antibody. Nonetheless, direct cell contact is required for cell death as shown in figure 1-9. The cytotoxic T cell receptor binds to the target antigen and the MHC class I molecule. It then secretes cytotoxins which causes the target cell to swell and self-destruct. After killing one target cell, the cytotoxic T cell can attach to another target cell and destroy that cell.

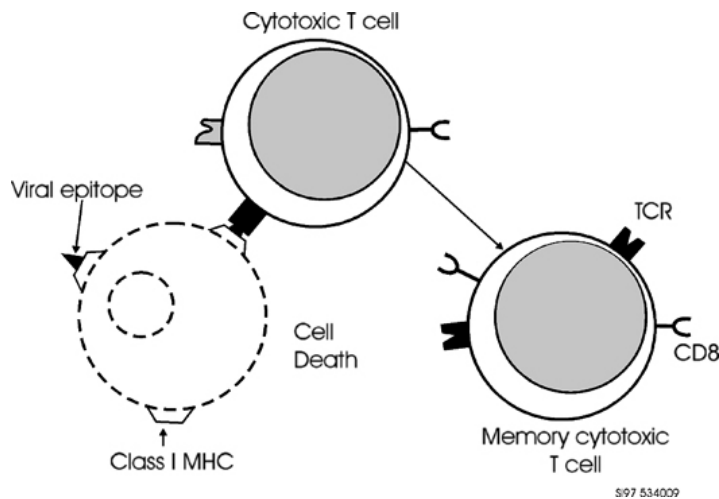


Figure 1-9. Cytotoxic T cell activation sequence.

### *B cell activation*

B cell activation results in proliferation and differentiation shown in figure 1-10. IL-4 (B-cell growth factor 1) stimulates the proliferation of antigen-activated B cells. IL-6 (B-cell growth factor 2) induces differentiation of proliferating B cells. Plasma cells are specialized, fully differentiated cells capable of synthesizing and secreting antibody of the same specificity as that of the receptors on their precursor surface membranes. At the same time, a proportion of daughter cells, produced in this



proliferative response, transform back into resting, mature memory B cells that are capable of being activated from a subsequent and even more rapid response.

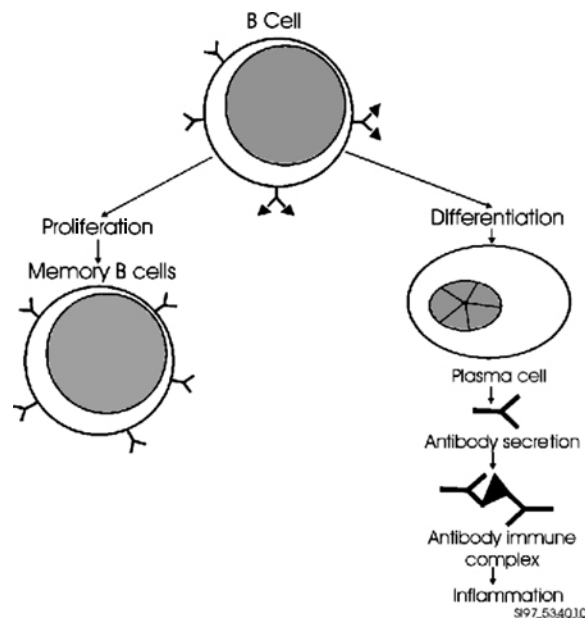


Figure 1-10. B cell activation sequence.

### Antibody production for the elimination of foreign invaders

The ultimate function of the immune system is to pursue and eradicate foreign substances in the body. This function is carried out by various means. The cytotoxic killing of antigen-bearing target cells mentioned above is one method. Most methods require antibodies. The most important antibody mechanisms are toxin neutralization, virus neutralization, opsonization and phagocytic actions, and activation of complement. These were described earlier under lesson 202. In regards to antibody production and testing, a study of the primary and secondary immune responses is crucial.

#### Primary antibody response

Before producing antibodies, B cells usually interact directly with the antigen and with T cells. Helper T cells, which have been activated by antigen presenting cells, produce cytokines (IL-4 and IL-6) which help B cells produce antibodies. Some large molecules, especially those with multiple repeating polysaccharide antigenic determinants, can induce antibody production without intervention from T cells, and are called *T cell-independent* antigens. In a *primary immune response*, when the host first encounters a particular antigen, several days to several weeks elapse before detectable antibody is found; this is called the *lag phase*. During this period, the B cell undergoes reorganization of gene material, assembly of ribosomes, initiation of DNA synthesis and, ultimately, mitosis. This is a key aspect of the immune response because if DNA synthesis and mitosis are disturbed, the amount of antibody produced is drastically reduced. Suitably stimulated by contact with antigen, B cells undergo clonal expansion and produce daughter cells that are genetically identical. The daughter cell may either develop into a plasma cell, the cell that actually secretes antibody, or become a memory B cell, which enters the circulating lymphocyte pool and persists for months or years. In the lag phase, no antibody is detectable. The *exponential* or *log phase* is marked by a rapid increase of antibodies in which the antibody titer rises logarithmically. During the *steady state* or *plateau phase*, the antibody titer stabilizes. The *declining phase* is so called because antibodies are rapidly degenerating and the antibody titer declines. Antibodies produced in the primary response are predominantly IgM. See figure 1-11 for an illustrated example of these phases.

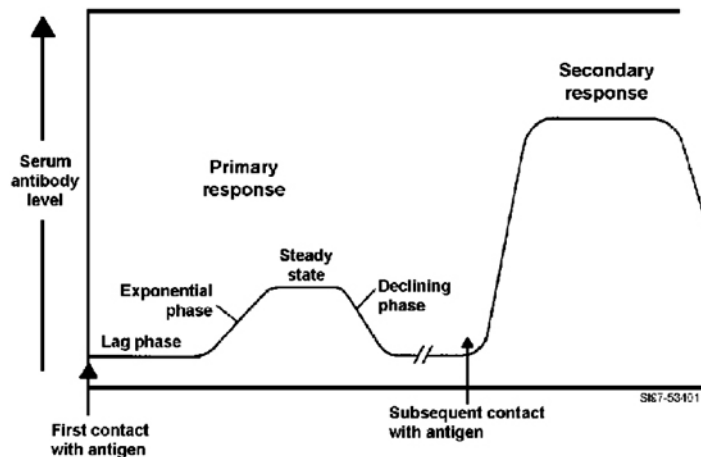


Figure 1-11. Illustration of primary and secondary antibody responses.

### ***Secondary antibody response***

It is the B cell that responds to subsequent contact with antigens to produce the secondary response. The *secondary* or *anamnestic* (memory), response occurs very rapidly and results in greater antibody production than in the primary response shown in figure 1-11. This is because memory B cells have already been exposed to the antigen and have produced daughter cells that will recognize that antigen. On a subsequent encounter with the same antigen more cells are able to respond. IgG is the major class of antibody produced in a secondary response.

## **205. The antigen-antibody complex**

The antigen-antibody interaction may well be explained as a lock and key concept. The lock (antigen) has tumblers (epitopes) that have dimensions. The key (antibody) has grooves (combining sites) that fit exactly into the tumblers of the lock. When the key is inserted and turned, the door opens (the interaction takes place). This may be an over-simplification of a very complex chemical reaction, but it is an illustration to clarify the actual combining of the antigen and antibody. When an antibody combines with its complementary or homologous antigen (the antigen that induced antibody production), an immune or antigen-antibody complex is created.

### **The complex**

The antigen-antibody complex may be small or large depending on the nature and dimensions of the antigen and antibody. Antibodies can react with antigens that are fixed (localized) in tissues or present in the circulatory system. Once the antigen-antibody complex is formed within the circulatory system, it is removed through phagocytic cells, interaction of the Fc portion with complement, and/or activation of cell-surface receptors. This is the body's major defense against the invasion of foreign substances. Under normal conditions, this doesn't lead to any pathological consequences, but under unusual circumstances the antigen-antibody complex escapes phagocytosis. It then persists as a soluble complex in the circulation where it can be carried to and deposited in different organs where it causes inflammatory damage. Detection of the antigen-antibody complex is vital to the clinical diagnosis of certain disorders.

### **Antigen-antibody reactions**

Antigen-antibody reactions can take place *in vivo* or *in vitro*. *In vivo* reactions usually interfere with the invasion of foreign antigens and in so doing protect against disease. In autoimmune states, *in vivo* antigen-antibody reactions may actually cause disease such as acquired hemolytic anemia or idiopathic thrombocytopenic purpura. The antigen-antibody reactions that the laboratory technician

observes are *in vitro* reactions. These reactions are of great interest and importance to the technician. It is through these reactions that antigens or antibodies can be detected and studied.

### ***Antigen-antibody binding forces***

When an antibody and its homologous antigen combine, they interact through chemicals found on their surfaces. Specifically, certain atoms or groups of atoms on the antigen combine with complementary atoms on the antibody. The antigen-antibody complex can be separated or reversed. The binding or reaction depends on the chemical nature of each reactant (antigen and antibody) and the forces that keep them together. The forces that hold antigen-antibody complexes together are explained in the following table.

Force	Explanation
Hydrophobic bonds	Hydrophobic bonds are the major bonds between antigens and antibodies. These bonds are related to the attraction between nonpolar groups, which exclude water molecules from the area of interaction.
Hydrogen bonds	Hydrogen bonds are the result of the formation of hydrogen bridges through the attraction of two negatively charged atoms of hydrogen.
Van der Waals forces	Van der Waals forces result from the mutual polarization of the external electron clouds of the two atoms.
Ionic or electrostatic forces	Electrostatic forces are the attraction of a positively charged portion of a molecule for a negatively charged portion of another molecule.

### ***Reaction sites***

Reactions can take place at a single site or many sites. As shown in figure 1-3, the basic 4-chain immunoglobulin (antibody) molecule is three-dimensional. Terminal domains in any one molecule are identical. Hence, it can react with one epitope or multiple epitopes at once. Although, the epitope the immunoglobulin reacts with, is identical.

### ***Goodness of fit***

The binding forces between antigens and antibodies are generally weak; so the antigen and antibody must be brought close together before the reaction occurs. The strongest bonding develops when antigens and antibodies are close to each other and when the epitope and combining site are the same shape and conform to each other. This conforming or complementary matching is called *goodness of fit*. An example is shown in figure 1-12. A good fit creates ample opportunities for the aforementioned binding forces to go to work. If a poor fit is present, repulsive forces can overpower any small forces of attraction. Goodness of fit determines the affinity of an antigen-antibody complex. If there is a good fit there is high affinity and if there is a poor fit there is low affinity.

### ***Affinity***

Affinity is the strength of the bond between a single (monovalent) epitope and an individual antibody combining site. However, most naturally occurring antigens and antibodies are poly- or multivalent (numerous epitopes and combining sites). IgM antibodies, due to their multiple combining sites, have high affinity.

### ***Avidity***

Avidity is the term used to denote the overall binding strength between multivalent antigens and multiple antibodies. It is the cumulative binding strength of all antigen-antibody combinations and therefore is related to the affinity of single epitope combinations.

### ***Specificity***

Specificity is the ability of a particular antibody to react with one antigen instead of another antigen. In other words, the antibody combines with the homologous antigen and is *specific* for that antigen. See figure 1-13 for an illustration.

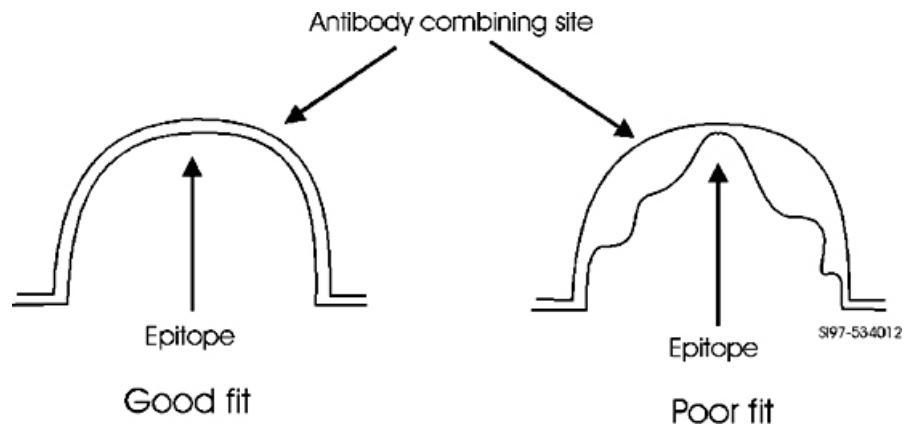


Figure 1-12. Goodness of fit.

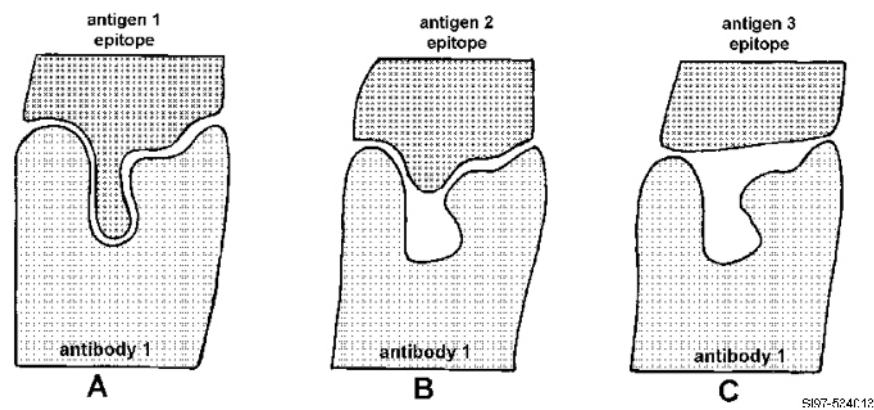


Figure 1-13. Examples of (A) high-specificity, (B) cross-reactivity, and (C) no reaction or combination.

### Cross-reactivity

Normal antigen-antibody reactions involve the specific coupling of epitopes with complementary combining sites on an antibody, which is usually polysaccharide in nature. In the case of cross-reactivity, two epitopes are so similar in structure that they react with a common antibody combining site. The greater the similarity between the cross-reacting antigen and the homologous antigen, the stronger the bond. No reaction occurs when an antibody can't combine with a dissimilar antigen.

### Uses of cross-reactivity

Many medically important microorganisms have either somatic or capsular polysaccharide substances as part of their chemical makeup. The use of the cross-reacting phenomenon simplifies very difficult testing, especially among those viruses and bacteria, which share a common antigen.

### Serologic test

The first serologic test used to diagnose human disease was for the detection of agglutinins in the serum of typhoid fever. These sera-agglutinated suspensions of the typhoid bacillus (*Salmonella typhi*) are employed by the Widal reaction (named after one of the early discoverers of this phenomenon). Cross-reactivity is easily exemplified with the rickettsial diseases, such as typhus and Rocky Mountain spotted fever. These rickettsiae have some antigens in common with certain bacteria

of the genus *Proteus*. The cross-reaction between the polysaccharides of certain strains of *Proteus* organisms and antibodies directed against certain *Rickettsiae* organisms is called the “Weil-Felix reaction.” This reaction is very useful in diagnostic tests for rickettsial diseases. Since bacteria are more readily cultivated than rickettsiae, the economic route is selected by using bacterial antigens (*Proteus* species) in serologic testing for the unrelated rickettsial diseases.

### Complement

It is difficult to analyze antigen-antibody reactions without discussing *complement*. So what is the role of complement in antigen-antibody interactions and in immunity? It is a complicated one, but, nonetheless, it deserves a brief discussion. To acquire a basic understanding, we need to trace a bit of its history and look into its properties and functions.

### History

Early bacteriologists noticed that blood from specific animals, when mixed with pathogenic bacteria, would kill the bacteria. However, only certain kinds of bacteria were destroyed, and the power to destroy was present only in the blood of animals immune to that particular type of bacterium. This *lytic* substance was found in serum as well as in whole blood or plasma. The lethal property was destroyed if the blood was heated to about 56°C, for 30 minutes. The *Pfeiffer reaction* demonstrated that if a test serum was heated to destroy this substance and then mixed with a small amount of normal guinea pig serum, the test serum would again destroy the microorganisms.

See figure 1-14 for an example of the lytic qualities of complement. It was concluded that the lytic substance was sensitive to heat and that some other heat resistant component (antibodies) in the serum was also necessary for the destruction. Bacteriolysis was not the only type of reaction this substance was responsible for. It was further demonstrated that this same lytic substance would cause hemolysis with an antiserum prepared against certain cells. Cytolysis of both bacteria and blood cells' is the direct result of an antigen-antibody reaction in the presence of the lytic substance, complement. However, complement itself does not possess the ability to lyse non-sensitized cells.

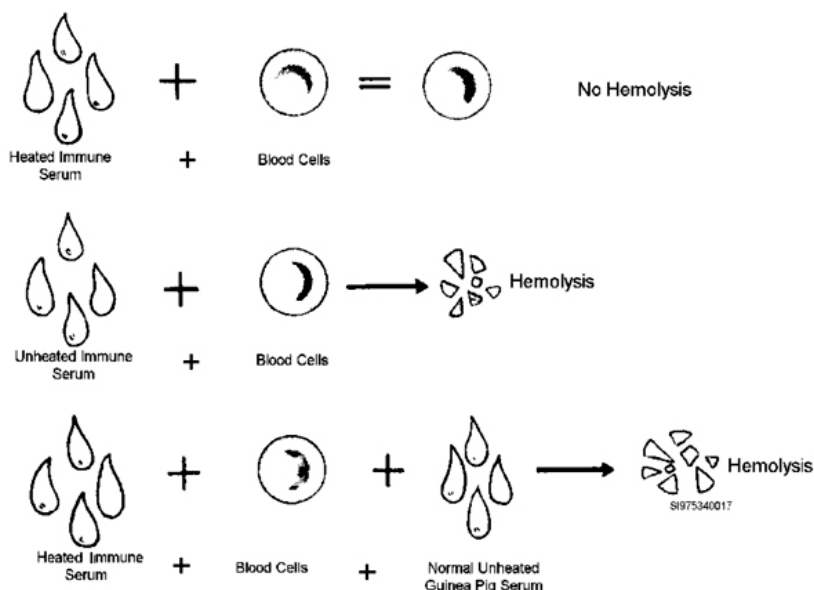


Figure 1-14. Lytic qualities of complement.

### Properties

Complement is a collective term used to denote a group of at least 20 different serum glycoproteins, most of which circulate as an inactive precursor form and develop proteolytic activity upon activation. The molecular weights of these components range between MW 71,000 and MW 410,000.

Unlike immunoglobulins, their concentration in serum doesn't increase after immunization. Complement proteins undergo activation either by attaching to or associating with other proteins, and/or by undergoing proteolytic cleavage (removal of part of the molecule) that leaves the residual portion enzymatically or biochemically active. The activation of complement and the products formed during the complement cascade have a variety of physiologic and cellular consequences. The sequence whereby each component undergoes activation and then activates the next component is often described as an amplifying enzymatic cascade. Complement is composed of two interrelated enzyme cascades; the *classic* and *alternative activation pathways*. **NOTE:** The complement pathways can be compared to the coagulation pathways in order to make them more understandable.

#### *Classic activation pathway*

Proteins of the classic activation pathway and the terminal sequence (common final pathway) are called components and are designated by the letter "C" followed by a number. The classic activation pathway is composed of C1 (C1q, C1r, and C1s), C4 and C2. They are named by their order of discovery and not by their order in the cascade sequence. The classic pathway requires antigen-antibody complexes for initiation but the alternative doesn't. The antibodies can be either IgG or IgM. The classic pathway is the primary amplifier of biologic effects of humoral immunity.

#### *Alternative activation pathway*

Proteins of the alternative activation pathway are called factors and are designated by uppercase letters. The alternative activation pathway consists of Factor B, Factor D, and Factor P (properdin). The alternative pathway of complement activation may be triggered by bacterial lipopolysaccharides (endotoxins), cell walls of certain bacteria and yeast, some virus-infected cells, aggregated IgA, and a factor from cobra venom. The alternative pathway amplifies nonimmune defenses.

#### *Terminal sequence or common final pathway*

Both pathways lead to the terminal sequence. The components of the terminal sequence are C3, C5, C6, C7, C8, and C9. The activation and assembly of complement components C5 through C9 constitute the cytolytic complex of the complement system. Both pathways convert C3 to C3b: which is the central event of this sequence. This in turn leads to the activation of the lytic complement sequence C5 through C9 and cell destruction. Both pathways can be divided into three simple units; (1) recognition, (2) enzymatic activation, and (3) membrane attack.

#### **Functions**

Complement plays a major role in the immune system as a potent mediator of inflammation. Its best known function is to cause lysis of cells, bacteria, and enveloped viruses. It also mediates the process of opsonization and regulates features of the inflammatory and immune responses. Complement is involved in many different types of activities. Some are reviewed below.

	<b>Complement Activity</b>
Anaphylatoxin	Anaphylatoxin is a substance, which mediates inflammation by inducing the release of histamine from basophils and mast cells by causing smooth muscle contraction and by increasing vascular permeability. It is associated with allergic reactions.
Immune adherence	Immune adherence is a phenomenon that facilitates the removal of soluble antigen-antibody complexes. It provides a mechanism for a soluble complex to adhere to vessel walls or erythrocytes, facilitating removal of the complex by the mononuclear phagocytic system (MPS).
Chemotaxins	Chemotaxins are substances that induce the direct migration of neutrophils and monocytes into the area of inflammation.
Opsonization	Opsonization refers to the coating of a particulate antigen by complement that renders the particle more attractive to the phagocytic cells for more effective phagocytosis by polymorphonuclear neutrophils or monocytes.

	Complement Activity
Lysis	Lysis is used in laboratory studies for <i>in vitro</i> activation of complement to measure the degree of sheep red blood cell lysis. It is also involved in antibody-mediated transfusion reaction and as a defense against <i>Neisseria</i> infections.
Kinin C2b	Kinin C2b interacts with plasmin to produce kinin-like activity which results in smooth muscle contraction, mucous gland secretion, increased vascular permeability, and pain.

### Complement-fixation

When antigens and antibodies react in the presence of complement, the complement is actually bound or fixed. It is unavailable for another reaction if other antigens or antibodies are later added to the reaction mixture. This binding effect is called *complement-fixation*. Fixation of C1q complex occurs when the C1q subcomponent binds directly to an immunoglobulin molecule. A single IgM molecule is potentially able to fix C1, but at least two IgG molecules are required for this purpose as shown in figure 1-15. **NOTE:** Figure 1-16 is not drawn to scale; C1q is enlarged to emphasize its structure. Remember the MW of IgG is MW 150,000, IgM is MW 900,000 and complement is between MW 71,000 and 410,000. Complement becomes fixed not only in bacteriolytic and hemolytic reactions but also in other antigen-antibody reactions as well. Some of these reactions do not give macroscopically observable results. Nevertheless, the reaction takes place and may be demonstrated by using blood cells sensitized with an appropriate antiserum as an indicator system. Various antibodies fix complement but some do not. If complement is present and an antibody reaction takes place, it becomes attached to the antigen-antibody complex. However, the quantity of the complement may be insufficient to cause cell lysis. We know that some antigen-antibody reactions are complement dependent. This can be demonstrated by studies in complement inactivation. Cells coated with complement-fixing antibodies are removed from the circulation much faster than cells sensitized with non-complement binding antibodies.

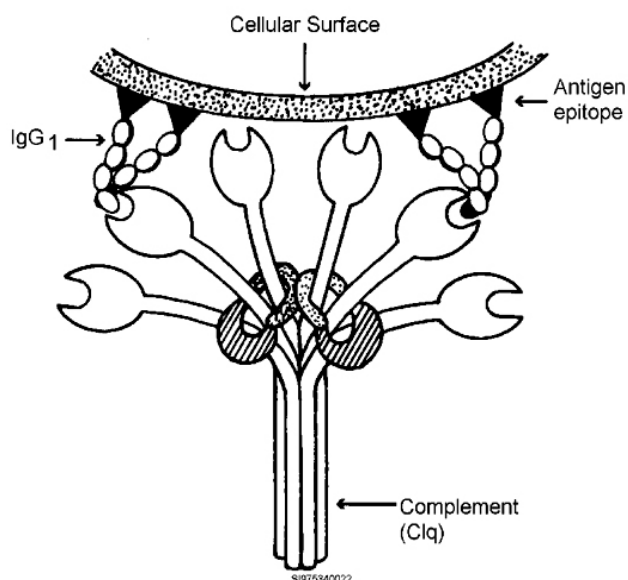


Figure 1-15. Complement fixed to IgG.

Complement occurs in the serum of most animals, but, the guinea pig is the usual source for testing purposes due to the more uniform yield of reliable product. In order to standardize our serological procedures as much as possible, complement from a single species is necessary.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 204. Antibody production

1. What are the *primary* cells involved in the immune response?
2. What is the function of the neutrophils?
3. What is the *most* important role macrophages play in the immune response and how do they do this?
4. What are effector cells?
5. How do T cells detect the presence of foreign substances?
6. What is the difference between helper and suppressor T cells?
7. What are cytotoxic T cells?
8. What is the capacity of B lymphocytes and into what do they differentiate?
9. What are natural kill cells?
10. What is step 1 in the production of antibodies?
11. What is step 2 and step 3?
12. What is the ultimate function of the immune system?
13. What are the phases in the primary immune response?
14. What is the difference(s) between the primary and secondary immune responses?



**205. The antigen-antibody complex**

1. What is the body's major defense against the invasion of foreign substances?
2. What are the antigen-antibody binding forces?
3. What is the definition of goodness of fit?
4. What is affinity?
5. What is avidity?
6. What is specificity?
7. What is cross-reactivity?
8. What is the definition of complement?
9. How do complement proteins undergo activation?
10. What are the two complement pathways of the complement cascade?
11. How would you describe the classic activation pathway?
12. How would you describe the alternative activation pathway?
13. What are the functions of complement?

## Answers to Self-Test Questions

### 201

1. A nonspecific immunity, possessed by a certain animal species due to some special property of that species, without the production of an antibody.
2. Skin, phagocytes, mucus, gastrointestinal tract, urinary tract, eyes, and lymphatic system.
3. Lysozymes, interferon, properdin, inflammation, complement, acute-phase proteins, and opsonins.
4. Adaptive immunity is an acquired or learned type of immunity; *humoral-mediated immunity* and *cell-mediated immunity*.
5. When the body recognizes a foreign substance and then produces antibodies against the specific substance this is known as humoral-mediated immunity.
6. Cell-mediated immunity consists of T lymphocytes that regulate antibody reactions by helping or suppressing the activation of B lymphocytes.
7. To acquire active immunity by natural means involves contracting a disease and thereby producing an antibody to that disease and artificially acquired active immunity is through vaccination.
8. Naturally acquired passive immunity is from antibodies that pass from the immune mother to the fetus and is relatively short-lived immunity; and artificially acquired passive immunity is from antibodies that have been produced in another individual or animal and then administered by injection.

### 202

1. An immunogen is a substance, which will elicit an immune response when introduced into the tissues of a normal, immunocompetent individual or host.
2. The specific set of chemical properties that is acknowledged by a given antibody or T cell receptor is called an *antigenic determinant* or *epitope*.
3. The degree to which a substance may produce an immune response is referred to as its *immunogenicity* or *antigenicity* (for antigens).
4. Molecular size, chemical composition and complexity, genetic composition of the host, and the method of administration.
5. Haptens are low molecular weight substances that can combine with antibodies but not evoke an immune response.
6. Antigen refers to the ability of a substance to combine with an antibody or T cell receptor.
7. Carbohydrates and proteins.
8. Blood group antigens and the major histocompatibility complex.
9. The application of the MHC is significant in organ transplantation, bone marrow transplantation, disease association, parental or family studies, forensic medicine, and in the regulation of the immune response during antigen presentation.

### 203

1. Specificity and biological activity.
2. Neutralization of toxins and viral activity binding, immobilization of microorganisms, agglutination of microorganisms or antigenic particles, binding with soluble antigens, activating serum complement, and activating phagocytic cells.
3. (1) They bind to specific antigens and (2) initiate a variety of secondary immune responses.
4. Heavy (H) chain and light (L) chain; light chains contain 2 of these domains and heavy chains contain 4 or 5 domains each.
5. Immunoglobulins can be split into 3 similar fragments: two identical monovalent antigen-binding fragments (Fab fragments) and one crystallizable fragment (Fc fragment).
6. The Fc fragment can't bind to an antigen but it is recognized by the Fc receptor on different cells.
7. Kappa ( $\kappa$ ) and lambda ( $\lambda$ ); alpha ( $\alpha$ ), delta ( $\delta$ ), epsilon ( $\epsilon$ ), gamma ( $\gamma$ ), and mu ( $\mu$ ).
8. The heavy chains are designated IgG ( $\gamma$ ), IgM ( $\mu$ ), IgA ( $\alpha$ ), IgD ( $\delta$ ), and IgE ( $\epsilon$ ).
9. IgG; peripheral blood, lymph, cerebrospinal, and peritoneal fluids.

10. IgM; pentamer macroglobulin that is composed of 5 basic 4-chain units with 5 globular domains ( $V_H$  and 4  $C_H$ ) on the heavy chains.
11. These antibodies are found in bodily secretions: saliva, tears, and fluids of the respiratory and gastrointestinal tracts. It is also the major immunoglobulin in the colostrum.

## 204

1. The cells primarily involved in the immune response are neutrophils, monocyte-macrophages, and lymphocytes.
2. They adhere to the blood vessel wall, migrate to the affected area, release chemical mediators and begin the process of engulfing any bacteria, cellular debris, or foreign particulate matter present at the site.
3. The most important role is their ability to control the action of lymphocytes; (1) by secreting potent immunoregulatory peptides [*tumor necrosis factor* (TNF) and *interleukin-1* (IL-1)] that control lymphocyte proliferation, differentiation, and effector function; and (2) acting as antigen-presenting cells.
4. Effector cells are cells that actively carry out an immunologic attack.
5. T cells don't express immunoglobulins, but detect the presence of foreign substances by the way of surface proteins called *T cell receptors*.
6. Helper cells promote the effector activities of both T and B cells, and stimulate macrophage activities and suppressor cells inhibit T and B cell activities.
7. Cytotoxic T cells are a subpopulation of T cells that destroy foreign or altered cells by direct cell-to-cell contact without the presence of an antibody.
8. B lymphocytes have the capacity to synthesize the heavy and light chains described earlier and to assemble them into immunoglobulin molecules; and B cells differentiate into memory B cells or plasma cells.
9. Natural killer cells are a group of lymphocytes that contain cytoplasmic granules and are capable of destroying virus-infected cells, neoplastic or allogenic cells.
10. Once the antigen enters the host it is bound to the APCs and certain antigens remain on the surface and are presented to specific lymphocytes.
11. T cell receptor recognizes and binds to that particular peptide-MHC complex and the helper T cell then becomes activated; the activated helper T cell begins to secrete cytokines for the proliferation and differentiation of T lymphocytes and B cells.
12. The ultimate function of the immune system is to pursue and eradicate foreign substances in the body.
13. Lag, exponential or log, steady state or plateau, and declining phase.
14. Response occurs very rapidly and results in greater antibody production than in the primary response.

## 205

1. Once the antigen-antibody complex is formed within the circulatory system, it is removed through phagocytic cells, interaction of the Fc portion with complement, and/or activation of cell-surface receptors.
2. The forces that hold antigen-antibody complexes together are hydrophobic bonds, hydrogen bonds, Van der Waals forces, and ionic or electrostatic forces.
3. When the epitope and combining site are the same shape and conform to each other, or complementary matching.
4. Affinity is the strength of the bond between a single (monovalent) epitope and an individual antibody combining site.
5. Avidity is the term used to denote the overall binding strength between multivalent antigens and multiple antibodies.
6. Specificity is the ability of a particular antibody to react with one antigen instead of another antigen.
7. In the case of cross-reactivity, two epitopes are so similar in structure that they react with a common antibody combining site.
8. Complement is a collective term used to denote a group of at least 20 different serum glycoproteins, most of which circulate in an inactive precursor form and develop proteolytic activity upon activation.
9. Complement proteins undergo activation either by attaching to or associating with other proteins. Also, by undergoing proteolytic cleavage (removal of part of the molecule) that leaves the residual portion enzymatically or biochemically active.

10. The *classic* and *alternative activation pathways*.
11. The classic pathway requires antigen-antibody complexes for initiation; the antibodies can be either IgG or IgM; and it is the primary amplifier of biologic effects of humoral immunity.
12. The alternative pathway of complement activation may be triggered by bacterial lipopolysaccharides (endotoxins), cell walls of certain bacteria and yeast, some virus-infected cells, aggregated IgA, and a factor from cobra venom; and it amplifies nonimmune defenses.
13. It is a potent mediator of inflammation; its best known function is to cause lysis of cells, bacteria, and enveloped viruses; and it also mediates the process of opsonization and regulates features of the inflammatory and immune responses.

**Do the unit review exercises before going to the next unit.**

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## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

1. (201) What innate chemical defense mechanism process is intended to eliminate debris and repair damaged tissues?
  - a. Properdin.
  - b. Interferon.
  - c. Lysozyme.
  - d. Inflammation.
2. (201) When the body recognizes a foreign substance then produces antibodies against the specific substance, it is known as
  - a. cell-mediated immunity.
  - b. anatomical innate immunity.
  - c. humoral-mediated immunity.
  - d. artificially acquired passive immunity.
3. (201) An individual contracting chicken pox and subsequently developing immunity is an example of
  - a. naturally acquired active immunity.
  - b. naturally acquired passive immunity.
  - c. artificially acquired active immunity.
  - d. artificially acquired passive immunity.
4. (201) An individual vaccinated against smallpox and subsequently developing immunity is an example of
  - a. naturally acquired active immunity.
  - b. naturally acquired passive immunity.
  - c. artificially acquired active immunity.
  - d. artificially acquired passive immunity.
5. (202) The specific set of chemical properties that is acknowledged by a given antibody or T cell receptor is called
  - a. a hapten.
  - b. an epitope.
  - c. a nucleic acid.
  - d. a homopolymer.

6. (202) What immunogenicity factor, which is described by “large, macromolecular proteins and complex carbohydrates, provides the most potent immunogens”?
  - a. Molecular size.
  - b. Chemical complexity.
  - c. Chemical composition.
  - d. Genetic composition of the host.
7. (202) What group of antigens is important in the regulation of immune responses during antigen presentation?
  - a. Autoantigens.
  - b. Bacterial antigens.
  - c. Blood group antigens.
  - d. Major histocompatibility complex antigens.
8. (203) What two important characteristics do immunoglobulins have?
  - a. Specificity and biological activity.
  - b. Specificity and chemical complexity.
  - c. Molecular size and biological activity.
  - d. Molecular size and chemical complexity.
9. (203) Which one of the following classes of immunoglobulins makes up the largest class in the peripheral blood and can pass through the placental barrier?
  - a. IgA.
  - b. IgD.
  - c. IgG.
  - d. IgM.
10. (204) The cells *primarily* involved in the immune response are
  - a. basophils, monocyte-macrophages, and lymphocytes.
  - b. eosinophils, neutrophils, and monocyte-macrophages.
  - c. neutrophils, monocyte-macrophages, and lymphocytes.
  - d. basophils, monocyte-macrophages, and variant lymphocytes.
11. (204) Which one of the following protein molecules essentially transmits messages between cells?
  - a. Cytokine.
  - b. Complement.
  - c. Cell adhesion.
  - d. Cluster of differentiation.
12. (204) The *main* function of B cells is
  - a. secreting antibodies.
  - b. as an activating complement.
  - c. influencing growth of other cells.
  - d. acting as antigen-presenting cells.
13. (204) IgG is the *major* class of antibody produced in
  - a. a primary response.
  - b. a cytotoxic response.
  - c. a secondary response.
  - d. an innate immune response.

14. (205) What is it called when an epitope and combining site are the same shape and conform to each other?
- a. Avidity.
  - b. Affinity.
  - c. Specificity.
  - d. Goodness of fit.
15. (205) The ability of a particular antibody to react with one antigen instead of another antigen is called
- a. avidity.
  - b. affinity.
  - c. specificity.
  - d. cross-reactivity.
16. (205) The two interrelated enzyme cascades of complement are
- a. classic and intrinsic pathway.
  - b. extrinsic and intrinsic pathway.
  - c. classic and alternative pathway.
  - d. extrinsic and alternative pathway.
17. (205) The *best* known function of complement is
- a. as a potent mediator of inflammation.
  - b. it mediates the process of opsonization.
  - c. it induces the direct migration of neutrophils.
  - d. to cause lysis of cells, bacteria, and enveloped viruses.

**Please read the unit menu for unit 2 and continue ➔**

## Unit 2. Immunology Procedures

206. Safety, specimen collection, and quality control .....	2-1
207. Techniques.....	2-4
208. Methodologies .....	2-7
209. Procedures .....	2-12

**A**S A LABORATORY TECHNICIAN, you will use a wide variety of techniques and methodologies to perform testing procedures in immunology. The immunologic testing that you perform helps the physician by supporting the diagnosis and management of patients with immune disorders and infectious diseases.

This unit, “immunology laboratory environment,” refers to the overall physical and functional design of a workplace dedicated to the specific task of diagnostic clinical immunology. The work environment is clearly distinct from other work centers within a laboratory by virtue of the nature of the work performed.

Because of the exquisite specificity involved in the antigen-antibody complex, both *in-vivo* and *in-vitro*, this interaction is widely used for diagnostic purposes. The utilization of this interaction *in-vitro* is known as serology. The term immunoassay describes a procedure that uses the antigen-antibody interaction to detect the presence of an antigen, antibody, or an antigen-antibody complex. The basic principle of antigen and antibody reactions is used as the basis for many different types of testing procedures. This unit will also cover these techniques, methodologies, and procedures.

### 206. Safety, specimen collection, and quality control

In the immunology laboratory, precautions must be taken to prevent accidental exposure to infectious disease. This lesson is a general review of good laboratory safety practices, specimen collection, and quality control for the immunology section of the laboratory.

#### Safety

Safety is always an important subject and, by now, should be a way of life for all laboratory technicians. It is essential to keep safety practices in the forefront of all we do. Since the immunology section primarily test for infectious diseases, it is reasonable to believe that these diseases can be transmitted through the various body fluids being tested. Each infectious agent is different in its ability to be transmitted through the various body fluids. Most of the infectious agents and their transmission were explained in the *Microbiology*, 4T051B course. At this time, we will only briefly discuss human immunodeficiency virus (HIV) and hepatitis B virus (HBV) transmission. **NOTE:** Remember “Universal Precautions,” treat all specimens as if they were infectious!

#### *HIV and HBV transmission*

Although HIV is the most feared biohazard in the laboratory, HBV transmission is more probable and can also be fatal. In dried blood or serum, HBV can be stable at room temperature for up to 7 days. HIV can be infectious for up to 3 days at room temperature and longer if in an aqueous environment.

#### *Factors determining infectivity of HIV and HBV*

The factors determining infectivity of HIV and HBV include; (1) concentration of virus, (2) duration of contact, (3) presence of skin lesions or abrasions on health-care worker, and (4) the immune status of the health care worker from HBV vaccination. These viruses can directly enter the body by percutaneous inoculation, contamination of skin, exposure of mucus membranes, and through aerosols. Indirect entry can be through contaminated work surfaces or equipment. Occupational Safety and Health Administration (OSHA) “*Universal Precautions*” and/or Center for Disease

Control and Prevention (CDC) “*Standard Precautions*” must be followed at all times. HBV vaccinations are wise preventative measures for health-care workers. Presently, these vaccinations are mandatory for some AFSCs and mobility personnel in the Air Force.

### **Specimen collection**

After proper patient identification, collecting a proper specimen is the first step toward reporting accurate and reliable test results. The majority of immunology specimens are acquired through venipuncture. Proper specimen collection requires knowledge of the routine and special methods employed in immunology. Most immunology procedures are performed on serum. Nonetheless, check your laboratory’s operating instruction (OI) or reference (referral) laboratory guides for the appropriate specimen requirements for the ordered test. Specimens for immunology testing should be handled as aseptically as possible and stored at recommended temperatures. Most antigens and antibodies are highly sensitive to changes in temperature, oxidation, and proteolytic degradation.

#### ***Serum specimens***

If the specimen of choice is serum, be sure to allow the specimen to clot completely. Complete clotting may take up to an hour. Centrifuge according to OI and transfer serum into a clean, labeled, tube with a cap. If serum is contaminated with red blood cells, recentrifuge. Insure tube is tightly sealed. If the procedure is not performed immediately, refrigerate or freeze as directed by the appropriate OI. Repeated freezing and thawing should always be avoided. Split the specimen into different tubes if repeat or confirmatory testing is probable or required.

#### ***Specimen collection factors***

The following is a list of factors to consider when checking the adequacy of the specimen collected for immunology testing.

<b>List of Factors</b>
Know the sample type recommend by the manufacturer of the test kit. If plasma is the specimen of choice, check to see if the anticoagulant used will interfere with reagents.
Preparation of collection site and the collection procedure should be reviewed.
The equipment used to collect the specimen may cross react with the antigen or antibody to be analyzed.
The timing of acute and convalescent specimens collected during an infectious process.
What medications is the person taking? Certain medications can cross react with test reagents.
An adequate amount of specimen must be collect for repeat and/or confirmatory testing.
Proper patient identification and collection times are essential.

### **Quality improvement**

Quality improvement is just as important in immunology as it is throughout the laboratory. It should be an established integral part of our daily operation. Remember that quality improvement (QI) is a program intended to monitor all the steps or methods used to assure accurate and reliable results. This includes overall laboratory management and components of quality control. A meaningful quality improvement program must be able to monitor both quality control (QC) and other laboratory activities. The activities can be divided into two basic categories: (1) analytic QC and (2) nonanalytic control. Nonanalytic control encompasses all activities not directly associated with the performance of the clinical assay itself. This includes both preanalytical and postanalytical activities. Preanalytical activities are ordering test; ensuring correct patient identification and preparation; specimen collection, transport, identification, and accessing; and specimen processing for analysis. Postanalytical activities are reporting results, workload recording or test charging, and specimen storage. The same basic quality improvement measures should be followed in regards to equipment and instrument maintenance, temperature recording, quality control documentation (graphs, plots, charts, etc.), lot to lot correlations of test kits and controls, etc., as performed in other sections.



### ***Quality control***

Quality control in immunology is unique from most other sections of the laboratory in the area of antigen and antibody interaction. Because of this uniqueness, immunochemical testing and immunology quality control must address potential problems associated with antigen and antibody reactions.

### ***Areas of concern***

Your immunology quality control may need to address the following areas of concern.

- |   |   |
|---|---|
| 1. Antibodies must be consistent                    | Antibodies used to detect antigens in the specimen must be consistently directed towards the pathogens epitope(s). In other words, cross reactivity should be minimal.  |
| 2. Antigen and antibody concentrations vary widely  | Antigen and antibody concentrations vary widely from person-to-person, therefore, antigen and antibody excesses should be taken into consideration.   |
| 3. Antigen and antibody concentrations below limits | Antigen and antibody concentrations may be below detectable limits. For example; if the antigen level is low, it may be too early in the disease process. Also, if antibody levels are too low, it may be because the infection is so severe the immune system is overcome or the patient is on immunosuppressive drug therapy. |
| 4. Presence of IgG versus IgM                       | Presence of IgG versus IgM is important if the procedure is suppose to detect one or the other, or both. The manufacturer and the laboratory must document adequate performance of the test for IgG, IgM, or IgG and IgM concentrations.  |
| 5. Purification of an antigen is critical           | Purification of an antigen is critical when analyzing certain antibodies. Also, specificity, avidity, affinity, and cross reactivity should be consider for all procedures.   |
| 6. Specimen matrix differences                      | Specimen matrix differences (blood, urine, CSF, feces, etc.) may markedly affect test performance.  |

### ***Unique quality control requirements***

Quality control requirements, which are unique for a specific procedure, will be addressed in the lesson about that particular procedure.

### ***Sources of error***

The most common sources of error in immunology procedures are listed below. **NOTE:** Some of these errors can overlap into other sections of the laboratory as well.

Common Errors
Wrong patient drawn.
Specimen improperly labeled.
Use of wrong or inadequately mixed reagents, dirty glassware, or hemolyzed specimen.
Incorrectly prepared dilutions.
Wrong incubation temperatures and times.
Erroneous reading or interpretation of results.
Incorrectly recording results.
Using an inappropriate or wrong assay.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 206. Safety, specimen collection, and quality control

1. In dried blood or serum, how long can HIV and HBV be infectious?
2. What are the factors for determining HIV and HBV infectivity?
3. How can viruses directly and indirectly enter the body?
4. Why should specimens for immunology testing be handled aseptically and stored at recommended temperatures?
5. How can repeated freezing and thawing of specimens be avoided?
6. What are the two basic categories of a quality improvement activities?
7. What does immunology quality control need to address that is different from other sections in the laboratory?

### 207. Techniques

Before we tackle our primary objective, immunology testing, we must explain the basic techniques that are part of the framework of serology testing. The skills and techniques used in serologic determinations are a combination of techniques used in other sections of the laboratory, and some are used only in immunology.

#### Titers

Serum for the detection of antibodies should be drawn during the acute phase of the illness and again during the convalescent period. The convalescent period is usually 2 weeks later, but may be longer depending on the infectious disease. When reporting certain serological results, the expression *titer* is used. A central theory of serological testing is the manifestation of a rise in titer from the acute specimen to the convalescent specimen. Titer defined specifically for serology is the concentration of antibodies in serum expressed as the reciprocal of the highest dilution giving complete agglutination or maximum precipitation. In other words, a high titer indicates that a high amount of antibody is present in the specimen. Be aware that antibody levels may not correlate with the severity of infection, the degree of immunity, or the ability of the patient's immune system to mount an immunologic response.

## Dilutions

Antibodies are often quantified by making several dilutions of the specimen (usually serum) and allowing the dilute specimens to react with a constant volume of antigen. If the reaction is visible or observable, we will see the reaction immediately or within a few minutes.

### Preparing dilutions

The procedure for diluting a solution is performed in a mathematical progression resulting in a dilution, which is inversely proportional to the concentration of the substance being diluted. Therefore, our dilution technique must be standardized so that the terminology used to identify a given dilution is properly interpreted. When we say that our titer has been derived by using a *two fold* or *serial* dilution, it must be understood that each dilution reduces the concentration of the specimen by one-half. A 1:2 dilution contains one part specimen and one part diluent. Likewise, a 1:10 dilution represents one part specimen and nine parts diluent. Hence, a titer expresses the units or parts present in a total volume and is written as “a titer of 1:2 or 1:10.”

### Microtitration or microtiter technique

The equipment used to make dilutions may vary from slides with wells to test tubes. The choice of equipment is largely dependent upon the amount of final volume required. However, dilutions are usually more accurate when performed in test tubes instead of slides. One of the newer techniques is *microtitration*. The basic components are a Plexiglas or plastic sheet (drilled with a series of U-shaped or V-shaped wells) and calibrated droppers, loops, or pipettes. (Pipettes are usually the most accurate.) The plastic sheet is known as a microtiter plate. For this technique, use a pipette to deliver the required amount of diluent and specimen into the appropriate well on the microtiter plate. Different specimens can be added to separate wells in the first row. The dilution is made by transferring a specific amount from one well to another (in that row), mixing, and transferring the same amount to the next well. This is a very rapid technique and cuts down the time and equipment required to perform large numbers of specimen dilutions.

### Calculating a dilution

The simplest method of calculating dilutions is to divide the total volume of the mixture by the amount of serum contained. For example, if a tube contains 1 ml of saline and 1 ml of serum the total volume is 2 ml. Dividing 2 ml (total) by 1 ml (serum) gives you a 1:2 dilution, which is a titer of 1:2. If you are performing a two fold dilution the next dilution is 2 times the previous dilution. This is because you are dividing the previous dilution in half. See the table below for an example of a two fold dilution.

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8
Saline	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Patient serum or preceding dilution	1 ml	1 ml of 1:2	1 ml of 1:4	1 ml of 1:8	1 ml of 1:16	1 ml of 1:32	1 ml of 1:64	1 ml of 1:128
Final dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256

### Two fold dilution

The next table is an example of a two-fold dilution beginning with a 1:10 dilution.

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Saline	9 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Patient serum or preceding dilution	1 ml	1 ml of 1:10	1 ml of 1:20	1 ml of 1:40	1 ml of 1:80	1 ml of 1:160
Final dilution	1:10	1:20	1:40	1:80	1:160	1:320

*Ten fold dilution*

This table is an illustration of a ten-fold dilution

	<b>Tube 1</b>	<b>Tube 2</b>	<b>Tube 3</b>	<b>Tube 4</b>
Saline	9 ml	9 ml	9 ml	9 ml
Patient serum or preceding dilution	1 ml	1 ml of 1:10	1 ml of 1:100	1 ml of 1:1,000
Final dilution	1:10	1:100	1:1,000	1:10,000

*Finding the dilution*

Generally, once you find the dilution of the first tube, the others are simply calculated. For most infectious diseases, an increase in the person's titer of two doubling dilutions is considered diagnostic of a current infection. For example, a patient comes to the MTF with a fever, a rash, and states they were possibly exposed to measles. The clinician orders a rubella test. First, an acute specimen is drawn and two weeks later a convalescent specimen is drawn. The results of the rubella procedure reveal a titer of 1:4 for the acute specimen and a titer of 1:16 for the convalescent. This shows an increase of two doubling dilutions and is called a *four fold rise* in titer. A four fold rise in titer indicates a current infection.

*Cell suspensions*

Occasionally, an indicator system, usually a cell suspension, must be added to the dilutions to make the reaction visible. Cells from various sources may be used as an antigen source or as an indicator system in serologic tests. The type of cells selected depends on the type of antigen they carry and the type of antibody to isolate or quantify. Cells from humans, cows, sheep, guinea pigs, chickens, horses, and several other animals are routinely used. Usually, a suspension of these cells is prepared in saline or albumin. Other media may be required, depending upon the type of antibody involved. With the addition of an indicator system, the dilutions or titers may be calculated differently. In immunohematological or blood bank tests, the dilution or titer is usually calculated prior to adding the cell suspension. On the other hand, in serological procedures, the dilution or titer is usually calculated after the cell suspension, hemolysin, or complement is added.

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**Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

**207. Basic techniques**

1. When is serum for the detection of antibodies drawn?
2. What is the central theory of serological testing?
3. How are antibodies quantified and what is involved?
4. How is the procedure for diluting a solution performed?

5. What is a two fold or serial dilution?
6. What does a titer express and how is it written?
7. What are the basic components of the microtitration technique?
8. What are the advantages of the microtitration technique?
9. For most infectious diseases, what titer result is considered diagnostic and what is it called?
10. How are indicator cells selected?

## 208. Methodologies

There are a wide range of testing procedures that use antigens to detect antibodies *or* antibodies to detect antigens. Each procedure utilizes a slightly different methodology. The interaction of different antigens with their corresponding antibodies can result in: *agglutination* (if the antigen is particulate), *precipitation* (if the antigen is soluble), or *activation of complement*. All of these outcomes are caused by the interactions between polyvalent antigens and antibodies that have at least two combining sites per molecule. Soluble antigens are usually toxins and foreign proteins, while insoluble antigens are usually bacteria and tissue cells. (The word particulate means “insoluble particle” and may also be used in place of insoluble antigen.) Other methods fall under the umbrella of *labeling* or *ligand assays* which include radioimmunoassays, enzyme immunoassays, fluorescence immunoassays, and chemiluminescent immunoassays. This lesson will examine basic methodologies and discuss their applications.

### Agglutination

Agglutination is the cross-linking or lattice formation of a solid or particulate (insoluble antigen) with a corresponding antibody and is observed as clumping. In reverse agglutination the antibody is attached to a solid particle and is agglutinated by an insoluble antigen. Agglutination procedures specifically aggregate particulate matter: such as bacteria, cells, or synthetic matrices (e.g., latex beads). These methods usually depend on reactions where either antigens or antibodies are bound or are attached chemically or physically to the surface of inert particles. Visible agglutination (lattice formation) is the result of the interaction of bivalent or multivalent antigens and antibodies that have at least two combining sites per molecule and cross-link with each other. The lattice formation continues to grow and becomes macroscopically visible. If they don't cross-link there is no lattice formation and no visible agglutination. These reactions are represented in figure 2-1.

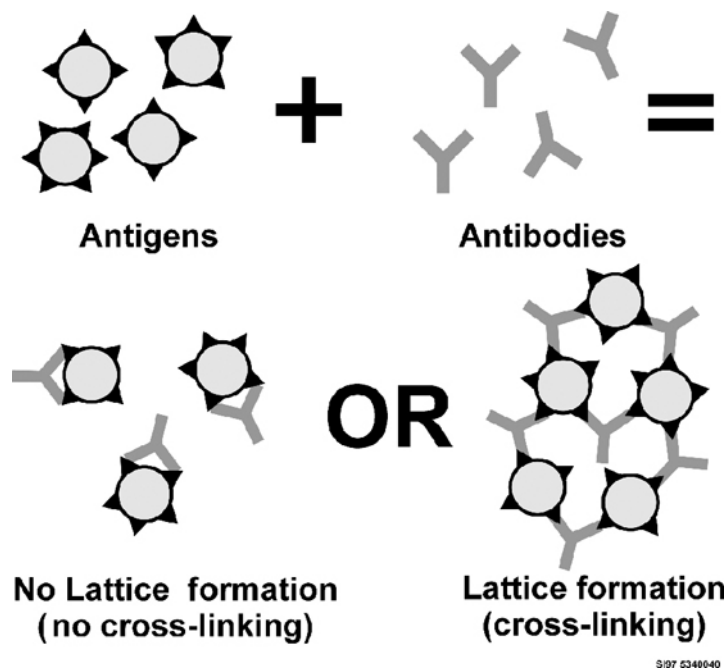


Figure 2-1. Schematic of a lattice formation.

### ***Where procedures used***

These procedures are used extensively in blood banking, immunohematology, microbiology, and in endocrinology for pregnancy testing.

### ***Factors affecting agglutination***

The major factors affecting agglutination include the size, class, affinity, and avidity of the antibody; number of binding sites; concentration of antibody and antigen; electrostatic interactions (zeta potential); and the type of medium or liquid used in the procedure. IgM antibodies have a total of 10 antigen combining sites and are considered the best “agglutinins” (up to 750 times more efficient than IgG).

### **Precipitation**

In contrast to the agglutination reaction (which takes place between antibodies and insoluble antigens) the precipitation reaction takes place when antibodies and soluble antigens are mixed in correct proportions. In these procedures, the reaction takes place between soluble reactants (antibody and antigen) to produce insoluble precipitates. Just like agglutination, precipitation occurs because multivalent antibody molecules cross-link multivalent antigen molecules to form a lattice. If serologically equivalent amounts of antigen and antibody are present a precipitate may form. Under conditions of antigen and antibody excess, small soluble complexes tend to predominate. When the antigen-antibody complex reaches a certain size, it loses its solubility and precipitates out of solution.

### ***Considerations***

Be aware that not all antibody complexes will precipitate. These procedures encompass immunodiffusion, immunoelectrophoresis, turbidimetry, and nephelometry. **NOTE:** Agglutination is the term used to describe the aggregation of insoluble or particulate test antigens, and precipitation is the term applied to aggregation of soluble test antigens.

### Optimal conditions

Under optimal conditions, there are equivalent amounts of antigens and antibodies in agglutination and precipitation procedures. Maximum precipitation occurs when the concentrations of antigen and antibody are in the zone of equivalence (serologically equivalent). Under conditions of antigen and antibody excess, small soluble complexes tend to predominate and there is no visible reaction. Let's look at the excesses.

### Prozone

Refer to figure 2-2. Prozone is an excess in antibody concentration and results in a diminished or absent detectable antigen-antibody complex. Also, there is no lattice formation.

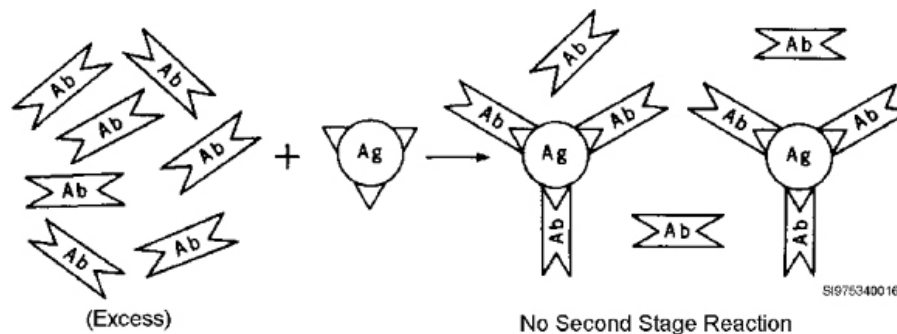


Figure 2-2. Illustration of a prozone reaction.

### Postzone

Refer to figure 2-3. Postzone is an excess in antigen concentration and results in a diminished or absent detectable antigen-antibody complex with no lattice formation.

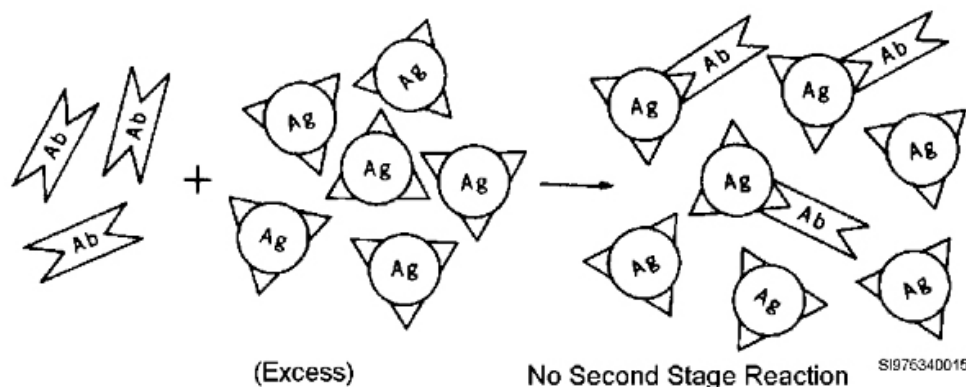


Figure 2-3. Illustration of postzone reaction.

### Complement assays

Complement assays can vary from actual measurement of the complement components and function to using complement as a reagent. Complement component and function assays will be studied in a different area. For this unit, we will look at using complement in the measurement of antigen-antibody complexes.

### Labeling techniques and ligand assays

Labeling techniques and ligand assays are usually interrelated. However, first you need to understand the labeling technique before moving on to ligand assays.

### Labeling techniques

The agglutination method may be considered the first labeling technique. It employs an inert particle that is chemically or physically attached to an antigen or antibody. In other words, the inert particle was *labeled* with an antigen or antibody. The label is an active molecule that is covalently bound to non-critical free amino or carboxyl groups or is added by other means to the molecule. The label allows the reaction to be measured, amplifies the power of the test, and increases its sensitivity. The characteristic of the label determines the procedure and detection mechanism. For example, if the label is a coated latex particle then the reaction may be agglutination and detected macroscopically or through light scattering (turbidimetry and nephelometry). On the other hand, if the label is a radioactive isotope, detection is accomplished by either a liquid scintillation counter for alpha or beta emitters, or a solid crystal gamma counter for gamma emitters. The labeled reactant can be in a liquid (soluble) or solid (bound) form. It may be bound to the surface or latex particles, plastic beads, test tubes, or microtiter plates.

### Ligand assays

The term ligand comes from the Latin word *ligare*, which means “to bind.” Dorland’s Illustrated Medical Dictionary defines ligand “as a molecule that binds to another molecule, used especially to refer to a small molecule that binds specifically to a larger molecule, for example, a hormone or neurotransmitter binding to a receptor, or a substrate or allosteric effector binding to an enzyme, or antigen binding to an antibody.” Examples of ligands are hormones, drugs, autoantigens, infectious agents, antibodies, lipoproteins, and tumor markers. These are the substances to be measured and can be known as a ligand, analyte, or hapten. Binding reagents (substrates) must have a specific configuration to a ligand. These reagents can be receptors, binding (carrier) proteins, or antibodies. See the table below for examples.

Type of assay	Type of binding reagent	Examples of analytes tested
Receptor	Receptor	Human chorionic gonadotropin, progesterone receptors, estrogen receptors
Protein binding	Binding proteins	T <sub>3</sub> , T <sub>4</sub> , cortisol, testosterone, estrogen
Immunoassay	Antibody	Rubella virus, Rubella antibody, HIV antibody, human chorionic gonadotropin

### Ligand assay reactions

As stated earlier, a ligand is a molecule that combines with specific complementary configurations of the binding reagent (receptors, proteins, or antibodies). To measure the amount of complex that is formed either the ligand or the binding reagent must be labeled. The general reaction is shown in the equation below.



### Earliest assays

The earliest assays used a labeled ligand (usually a radioactive isotope) known as a *tracer*. The amount of ligand that binds to the binding reagent is related to the number of binding sites and the affinity of the binding reagent. Reactions can occur in a soluble or solid phase. Some methods require a step to separate the free labeled reactant from the bound labeled reactant, other methods do not.

### Separation techniques

The different separation techniques are adsorption, nonimmune or chemical precipitation, immune precipitation, or the use of a solid phase support. Ligand assays are either heterogeneous or homogeneous. Heterogeneous assays require a separation of the free label from the bound label before quantitating the bound label. Homogeneous assays do not. Competitive binding assays and sandwich assays, to detect either an antigen or antibody, are considered heterogeneous ligand assays.



Homogeneous ligand assays are enzyme-multiplied immunoassay techniques (EMIT), enzyme-enhanced immunoassays, coenzyme labeled immunoassays, enzyme immunochromatography, substrate-labeled fluorescent immunoassays, and fluorescence polarization immunoassays (FPIA).

### Type of measurement

All of the above methods are either qualitative, semi-quantitative, or quantitative.

#### *Qualitative procedures*

Qualitative procedures report on the presence or absence of the analyte.

#### *Semi-quantitative procedures*

Semi-quantitative procedures are essentially qualitative assays with an additional option for a response range. This is done by the degree of positivity (for example, 1+ to 4+), diluting the specimen and retesting, or comparing results to a color chart.

#### *Quantitative procedures*

Quantitative procedures generate a spectrum of signal responses that correlate with the concentration of the analyte of interest. If analyte preparations of known concentrations are available for calibration, the actual concentration of the analyte can be determined and enumerated.

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## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 208. Basic methodologies

1. Match the terms in column B with the statement in column A. Each element in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
____ (1) An inert particle with an antigen or antibody chemically or physically attached.	a. Agglutination.
____ (2) Antibody is attached to a solid particle and is detecting an insoluble antigen.	b. Precipitation.
____ (3) Excess antigen.	c. Activation of complement.
____ (4) The result of the interaction of bivalent or multivalent antigens and antibodies.	d. Reverse agglutination.
____ (5) Size, class, affinity, and avidity of antibody, number of binding sites, and antibody/antigen concentrations.	e. Particulate matter.
____ (6) Report of presence or absence of the analyte.	f. Factors affecting agglutination.
____ (7) Aggregation of soluble test antigens.	g. Prozone.
____ (8) Either heterogeneous or homogeneous test.	h. Postzone.
____ (9) Methods requiring separation of free labeled reactant from bound labeled reactant.	i. Labeling techniques.
____ (10) Excess antibody.	j. Ligand assays.
____ (11) Immunodiffusion and immunoelectrophoresis assays use this method.	k. Qualitative procedures.
____ (12) Assays using dilute specimens or color chart comparisons.	l. Semi-qualitative procedures.
____ (13) Aggregation of insoluble or particulate test antigens.	m. Quantitative.
____ (14) A molecule that binds to another molecule; a hormone binding to a receptor.	n. Lattice formation.
____ (15) Bacteria, cells, and synthetic matrices.	
____ (16) Determining the actual concentration of an analyte.	

## **209. Procedures**

This section will discuss the basic procedures used in immunology. These procedures are covered together in one lesson in order to facilitate your overall understanding of the commonalities and specifications.

### **Agglutination**

Agglutination procedures may be qualitative or semi-quantitative with reactions occurring within minutes-to-hours. These can be performed on slides, in test tubes, or in microtiter plates. Procedures that use the agglutination methodology include direct agglutination, indirect or passive agglutination, hemagglutination, agglutination inhibition, and Coombs testing.

#### ***Direct agglutination***

Direct agglutination procedures use antigens in their native state and are found naturally on the surfaces of bacteria, fungi, or human cells. An example is the ABO and Rh systems found on human red blood cells that react directly with patient serum. Other examples include cold agglutinins, febrile agglutinins, and heterophile or infectious mononucleosis tests. Some direct agglutination procedures lack sensitivity and specificity, hence require other tests for confirmation.

#### ***Indirect or passive agglutination***

Indirect or passive agglutination procedures utilize particles as passive carriers of antigens. An antigen is attached to the particle, mixed with patient serum, and observed for agglutination.

#### ***Term used***

Some text use the term passive agglutination for this technique and indirect agglutination exclusively for the use of indirect anti-human immunoglobulin (Coombs) testing. Rapid plasma reagin, rheumatoid factor, rubella, and thyroglobulin tests are examples of passive agglutination procedures.

#### ***Reverse passive***

Reverse passive procedures use antibodies attached to the carrier particle instead of antigens; an example is the C-reactive protein test.

#### ***Hemagglutination***

Direct or indirect agglutination procedures using erythrocytes as the carrier particle are known as hemagglutination procedures. Certain viruses can agglutinate red blood cells by binding receptors on their surface. This is a natural occurring phenomenon with rubella and influenza viruses.

#### ***Agglutination inhibition***

Agglutination inhibition procedures use competitive binding in which the soluble antigen and the antigen-coated particle (insoluble antigen) carrier compete for the soluble antibody. If the carrier is a red blood cell then it is known as hemagglutination inhibition, and if the carrier is a latex particle then it is known as latex agglutination inhibition. This is usually a two stage procedure. In the first stage, the soluble antigen present in the patient's specimen is incubated with a known antibody reagent. The second stage consists of adding the insoluble antigen (carrier). If the antigen is present in the patient's specimen, it is bound to the antibody reagent, and there is no free antibody to bind to the added antigen-coated carrier. If there is no visible agglutination present, then agglutination was inhibited, and the result is a positive test. If the antigen-coated carrier and the reagent antibody combine, then you will see visible agglutination which means your have a negative test. HBsAg, factor VIII antigen testing for hemophilia, clotting disorder tests, and some pregnancy test use this procedure.

### ***Anti-human globulin or Coombs test***

In some agglutination reactions, the antigen-antibody complex will not form a visible lattice. The anti-human globulin procedure, by Coombs, is used to complete the antibody bridging and increase lattice formation resulting in visible agglutination. The next volume, *Blood Banking*, will go deeper into the Coombs procedures.

#### ***Direct antiglobulin (Coombs)***

The direct antiglobulin test detects antibodies that have attached to the patients red blood cells *in-vivo*. The direct Coombs test is used to detect hemolytic disease of the newborn (HDN) or drug induced autoantibodies or coated red blood cells.

#### ***Indirect antiglobulin***

The indirect antiglobulin is a two stage procedure used also to detect antibodies in specimens. In the first stage, patient serum is incubated with test red cells to allow the antibody to bind with the antigen. In the second stage, the antibody-coated cells are agglutinated with anti-human immunoglobulin or Coombs reagent. This procedure is used for the detection of red cell antigens or antibodies, in determining compatibility of blood, and diagnosing autoimmune hemolytic anemia.

### **Precipitation**

Precipitation tests are qualitative, semiquantitative, or quantitative, and can take hours-to-days to complete. In these procedures, the antigen-antibody reaction produces a soluble antigen-antibody complex, which results in a visible precipitate or flaky sediment in fluid or semisolid gel. In a gel medium, there may be a precipitation line or ring at the interface of the antigen and antibody reaction. *Immunodiffusion* tests are based on the migration of the antigen or antibody through a fluid or gel medium until optimum proportions of each are reacted and a precipitate appears. *Double immunodiffusion* is the term used when both the antigen and antibody diffuse through the medium. *Immunoelectrophoresis* procedures combine double immunodiffusion and electrophoresis techniques. Immunoelectrophoresis relies on the movement of an antibody toward the cathode and an antigen toward the anode in an electrical field.

#### ***Fluid phase immunodiffusion***

This double diffusion method uses a fluid medium in which an antigen solution is placed on top of an antibody solution within a capillary tube. After passive diffusion, the antigen-antibody complex precipitates in the zone of equivalence (the area where the antigen and antibody meet in optimal concentrations). This method can be used to detect either unknown antigens or unknown antibodies. It was one of the first precipitation test but is rarely used today.

#### ***Gel phase immunodiffusion***

In these methods, different types of gels are used as a semisolid medium that allows antigens and/or antibodies to diffuse through the pores until they reach the zone of equivalence.

#### ***Double immunodiffusion Ouchterlony method***

In this procedure, an antigen is placed in one well and an antibody in another well, diffusion (spreading) occurs, and where the two meet in the gel, a line of precipitation results from the lattice formation. More than one antibody or antigen specimen can be tested with the known antigen or antibody as shown in figure 2-4.

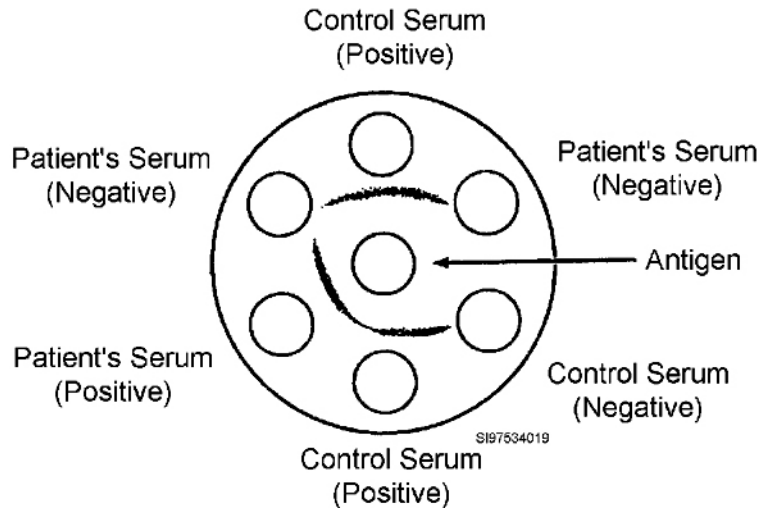


Figure 2-4. Example of the Ouchterlony double diffusion method.

#### *Single radial immunodiffusion (RID)*

In this procedure, the antibody is added to the liquefied gel and is poured into a plate and allowed to solidify. The antigen is added to wells cut into the gel. Then the antigen diffuses in all directions and a ring of precipitation forms in the zone of equivalence. The size of the ring is related to the concentration of the antigen as shown in figure 2-5.

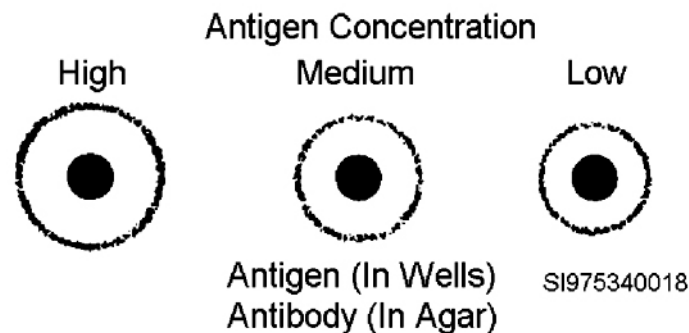


Figure 2-5. Example of a single radial immunodiffusion method.

#### *Countercurrent immunoelectrophoresis (CIEP)*

Both antigen and antibody are each placed into respective wells at the opposite end of a gel plate, away from the pole to which they will be drawn. The gel plate is placed in an electrical field and the antigen and antibody migrate toward each other. Precipitation will, once again, occur at the juncture of antigen and antibody interaction as they migrate to their designated electrical pole. This is a qualitative procedure, but, by using serial dilutions, it can be semiquantitative. If testing for an antibody, the antigen is at a constant concentration; and if testing for an antigen, the antibody is at a constant concentration.

#### *Rocket immunoelectrophoresis or Laurell technique*

In this procedure, a specific antibody is added to the agar or gel. The unknown antigen is added to a certain well and is electrophoresed. As the antigen and antibody interact, precipitation occurs in a tapered rocket-shaped pattern. The antigen concentration is directly proportional to the total distance or size of the rocket migration. Therefore, it is used for quantitation of antigens.

*Immunoelectrophoresis (IEP)*

As stated earlier, precipitation takes from several hours-to-overnight to appear. Faster results can be obtained by combining double immunodiffusion with protein electrophoresis to produce immunoelectrophoresis. This modification speeds up the reaction time and allows antibodies or specific globulin fractions in serum, urine, CSF, etc., to be separated. This procedure is considered qualitative and consists of two steps. First, specimens (or antigens) are placed in wells and are electrophoresed. After electrophoretic separation of proteins, anti-human serum (or antibody) is placed in a center trough that runs parallel to the wells. Immunodiffusion is allowed to occur with precipitin arcs appearing in the zone of equivalence.

*Immunofixation electrophoresis (IFE, IFX)*

Immunofixation electrophoresis is similar to immunoelectrophoresis, in that it is a two step procedure. It is used to identify genetic variants of proteins in serum, urine, and CSF. First, the specimen is electrophoresed and the proteins are separated. Then an antisera soaked material is applied directly to the surface of the gel (instead of added to a trough). Immunodiffusion occurs with a band of precipitation in the zone of equivalence.

***Turbidimetry and nephelometry***

Turbidity is the cloudiness of a solution caused by the scattering of light by colloidal particles or by suspended precipitate or sediment. Turbidimetry is the measurement of the turbidity of a solution. A turbidimeter is an instrument that measures the turbidity of a solution by measuring the loss of intensity of a beam of light passing through the solution. Nephelometry is also a way of measuring turbidity. A known amount of a specific antibody is added to a solution containing the antigen being measured. The intensity of light scattered, from the large antigen-antibody complexes formed during the reaction, is measured. As you can see from the above definitions, these techniques can be used for measuring the precipitation of antigen-antibody complexes.

**Complement fixation**

Complement fixation (CF) is a technique based on the principle that certain immune complexes are able to bind or fix complement. There are two distinct reactions involved in the complement fixation test. This first involves the use of a fixed amount of pre-titered complement plus an antigen and an antibody (one known, the other unknown). If the antigen and antibody are specific for one another, the combination will “fix” the available complement. The second reaction involves testing for any free or unattached complement left after the first reaction. Red cells, sensitized with a specific hemolysin, are added to the reaction media. If the complement has remained unfixed by the first antigen-antibody reaction, the red cells will lyse. Free complement left for the hemolysis of red cells would then indicate that the antigen and antibody were not specific for each other or one is lacking. The complement used is composed of eleven serum constituents, which become inactivated (or fixed) in many antigen-antibody reactions. A positive complement fixation reaction is then indicated by no hemolysis, while a negative reaction will show hemolysis.

**Ligand assays**

The ligand (analyte, hapten) is the substance to be measured in a ligand assay. The binder (binding reagent) is a receptor, protein, or antibody. In a ligand assay, one reactant (ligand or binder) must be labeled to measure the reaction. Assays are named according to the attached label: radioimmunoassays use a radioactive isotope label, enzyme immunoassays use an enzyme label, fluorescence immunoassays use a fluorochrome label, and chemiluminescence immunoassays use a light-emitting chemical label.

***Radioimmunoassay (RIA)***

Overall, this is the most sensitive technique available for quantifying biological substances that are in very low concentrations within the body. This method usually uses the gamma emitter  $^{125}\text{I}$  as the tracer. Some RIA procedures require a step to separate bound antigen or antibody from free antigen or antibody.

***Types of RIA procedures***

The two most common types of RIA procedures are excess reagent and competitive binding. We will only discuss competitive binding since the excess reagent method is rarely used in the clinical laboratory.

***Competitive binding***

Competitive binding is based upon competition for a specific antigen (antibody) between the known labeled antibody (antigen) and the unknown unlabeled patient antibody (antigen) being evaluated. The complexes formed are separated and the amount of radioactivity quantitated. The amount of radioactivity is measured and compared to the radioactivity of a series of known standards. The greater the amount of labeled antibody that is bound, the less unlabeled patient antibody is bound. In essence, this method measures the ratio of bound labeled antibody to unbound unlabeled antibody. In another variation, a fixed quantity of antigen is attached to a solid surface and a fixed quantity of indicator antibody, in solution, is allowed to bind to the antigen. The bound indicator antibody is measured using a radioisotope. The label may be attached directly to the indicator antibody or to a secondary antibody. At this time, there is a tendency to replace radioactive isotopes with enzyme, fluorescent, or light emitting chemical labels. This is due to the regulations on use and disposal of hazardous radioactive materials.

***Enzyme immunoassay (EIA)***

Enzyme immunoassay is a generic term used for any ligand assay in which the label is an enzyme, coenzyme, enzyme inhibitor, or fluorogenic substrate, and the binding reagent is an antibody. EIA assays can be used to detect antigens and antibodies, and for both tissue and soluble reactants. The product of the specific immune reaction is detected by the enzyme and substrate reaction. There is either a change in optical absorbance in the visible or ultraviolet range (spectrophotometer) or fluorometric change (fluorometer) depending on the nature of the enzyme/substrate reactants used. The enzymes used in the different assays must be stable, highly purified, specific for the substrate, and must not be changed by the other reagents. These techniques also display considerable sensitivity. EIA assays are either heterogeneous or homogeneous. Heterogeneous EIA procedures require a solid-phase support and separation step. There are a variety of solid-phase supports that include test tubes; microtiter plates; latex, agarose, or polystyrene beads or particles; magnetized beads; microparticles; membranes; and glass fiber paper or membranes. Enzyme-linked immunoabsorbent assay (ELISA) is an example of a heterogeneous procedure. Homogeneous EIA procedures don't require a separation because the enzyme activity is regulated by the antigen-antibody interaction; an example is the enzyme multiplied immunoassay technique (EMIT).

***Enzyme-linked immunoabsorbent assay***

Enzyme-linked immunoabsorbent assay (ELISA) is a general term used for heterogeneous enzyme immunoassays. As stated above, they require a solid-phase support. For antibody determinations, the known antigen is fixed to a solid-phase support and then incubated with test serum dilution. After incubation, the solid-phase support is washed, and incubated with an enzyme-conjugated (labeled) anti-human globulin. After incubation and washing, a specific substrate is added. After a short incubation, the enzyme activity is then measured. The color reaction is estimated colorimetrically or by another technique if a different reaction is observed. An illustration of this procedure is shown in figure 2-6. The amount of bound enzyme-conjugated antibody is equal to the enzyme activity.

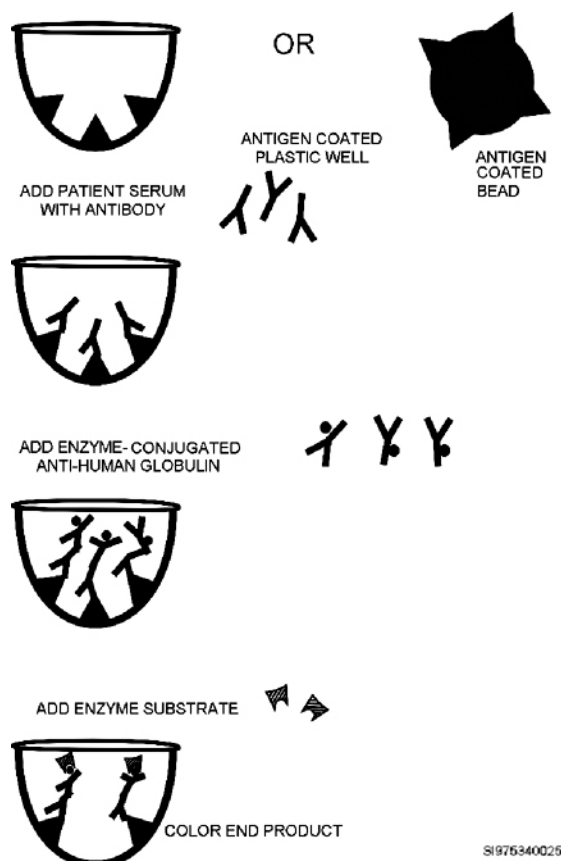


Figure 2-6. Illustration of an ELISA procedure for antibody determination

#### *Illustration of an ELISA procedure*

Antigen can be measured by fixing a known specific antibody to a solid-phase support, adding patient antigen, washing, and adding a second enzyme-conjugated antibody. See the illustration in figure 2-7. This test requires that the antigen have at least two determinants. A specific substrate is added after the second washing, and the enzyme activity is estimated colorimetrically.

#### *Enzyme-multiplied immunoassay technique (EMIT™)*

Enzyme-multiplied immunoassay technique (Syva Corporation) was the first homogeneous EIA procedure to be developed and is still used for the quantitation of drugs, hormones, and other biological substances. In the EMIT™ procedure the reactants include a ligand (hapten) that is covalently linked to an enzyme, an antibody directed against the ligand, and a substrate. In most methods, enzyme activity is inhibited through the binding of the enzyme-labeled ligand and antibody. The enzyme is catalytically active when the labeled ligand is free. When the antibody binds with the labeled ligand, it inhibits the catalytic activity of the enzyme. The unlabeled ligand (from the patient sample, standard, and control) and the enzyme-labeled ligand compete for the antibody binding sites. As the concentration of the unlabeled ligand increases, it combines with more antibodies. This decreases the amount of enzyme-labeled ligand from combining with the antibodies. Hence, the enzyme is not inhibited and can react with more substrate. The greater the amount of enzyme activity, the more labeled ligand that is free, and, in turn, the higher the concentration of unlabeled (patient) ligand. Essentially, the higher the enzyme activity the more drug, hormone, or other substance that is present in the patient sample.

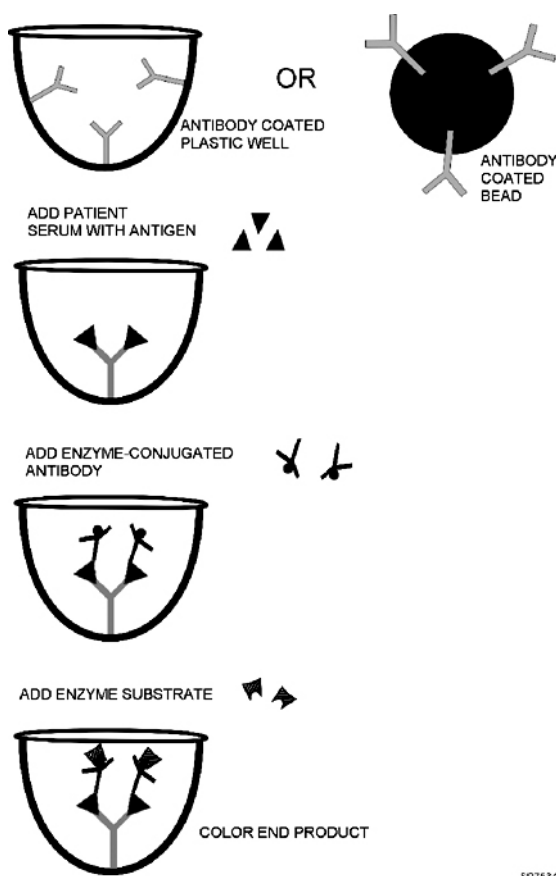


Figure 2-7. Illustration of an ELISA procedure for antigen determination.

### *Fluorescent immunoassays*

Fluorescence is a form of luminescence in which a molecule absorbs light energy of one wavelength and emits light energy of a longer wavelength. Fluorochromes (fluorescent dyes) are easily conjugated to most biological substances without changing the substance. For this reason, their application in the clinical laboratory increases. Nonetheless, the need for specialized instrumentation and purified, well-characterized reagents, their utilization is limited. As with RIA and EIA, fluorescent assays are heterogenous or homogenous and the labeled component can be a ligand or antibody.

### *Theory of fluorescence*

In order to comprehend fluorescence procedures, you must understand the properties of molecules in their ground (unexcited) and excited states. Molecules become excited due to changes that occur in their electron configuration. When molecules absorb electromagnetic radiation (energy) of proper wavelengths, the electrons are excited to higher electronic states or energy levels. Because these excited states are unstable, molecules dissipate this energy rapidly and return to the ground or resting state. Many molecules release this energy as heat and others release it in the form of photons (light) of electromagnetic energy. The energy of radiation is released in several wavelengths not a single wavelength. The emitted wavelength is at a lower energy level than the incident or absorbed light. Fluorochromes (e.g., fluorescein and rhodamine) have characteristic absorption and emission spectra. The emission process is based on the average length of time the molecule remains excited before re-emission of light. This time is referred to as the life time of the excited state. The two types of emissions, based on time of excitement, are fluorescence and phosphorescence. With fluorescence,



the re-emission of light is so rapid, that to the naked eye the re-emission appears to stop as soon as the exciting or incident light is removed. Phosphorescence emission can be observed for sometime after the removal of the exciting light. Basically, fluorescence is the emission of light of one color (wavelength), while a substance is irradiated with light of a different color. One of the major advantages of fluorescence is that it is 100 to a 1,000 times more sensitive than absorption measurements. However, limitations include background fluorescence particularly from autofluorescent substances normally present in serum, nonspecific binding of the reagents, fluorescence quenching (interference resulting in decreased fluorescence intensity), difficulties in differentiating the excitation and emission signals, and the light-scattering phenomenon. Fortunately, new fluorescent reagents and new techniques of applying these labels have alleviated many of these problems.

#### *Fluorescent microscopy*

Fluorescent microscopy is a modification of a darkfield microscope that uses special components to separate excitation wavelengths from emission wavelengths. In fluorescent microscopy, the exciting light, is often blue-green to ultraviolet. The light is provided by a high-pressure arc (mercury, xenon, or halogen) lamp. The color of emitted light depends on the nature of the substance. Fluorescein gives off yellow-green light and rhodamines fluoresce in the red portion of the spectrum. The color observed in the fluorescent microscope depends on the secondary or barrier filter used in the eyepiece. A yellow filter absorbs the green fluorescence of fluorescein and transmits only yellow. Fluorescein fluoresces an intense apple green color when excited. In practice, the actual brightness of fluorescence observed by the eye depends on these factors: (1) the efficiency with which the dye converts excited light into fluorescent light, (2) the concentration of the dye in the tissue sample, and (3) the intensity of the exciting (absorbed) radiation. In the future the small, inexpensive lasers that have recently become available in flow cytometers may be incorporated into the fluorescent microscope. They have longer life spans and produce monochromatic light of very high intensity.

#### *Direct fluorescent antibody technique (DFA)*

The fluorescent antibody technique consists of labeling antibody with fluorescein isothiocyanate (FITC), which has an affinity for proteins, to form a conjugate. This conjugate is able to react with antibody-specific antigens. These chemicals can be conjugated with antibodies without destroying antibody reactivity. For the *direct* reaction, a specific antibody is first conjugated with fluorescein. The labeled antibody is then reacted with its specific antigen, such as bacteria, spirochetes, or viruses. The mixture is then washed, leaving only those “tagged” antigens, removing excess antibody. This is represented in figure 2-8. The preparation (usually a slide impregnated with an antigen) is then read microscopically under ultraviolet light. Antigens emitting a fluorescence are indicative of a positive reaction. This technique is used mainly to detect an antigen in tissue or from culture by using known, labeled antibodies.

#### *Indirect fluorescent antibody technique (IFA)*

This modification of the fluorescent antibody technique is useful because it permits the detection of antibody in a patient's serum without the time-consuming process of labeling the antibodies of each patient with FITC. The unlabeled patient's serum is reacted with a known antigen (e.g., bacteria, spirochete, or virus) on a slide, incubated, and the excess antibody is washed away. In the second phase, anti-human globulin (directed specifically against IgM or IgG) conjugated to a fluorescent substance is placed on the slide. The labeled anti-human globulin reacts with the patient's antibodies attached to the antigens as shown in figure 2-8. When examined by a fluorescent microscope, fluorescence is indicative of a positive reaction. This test can be performed with serial dilutions of the patient's sample to determine the antibody titer.

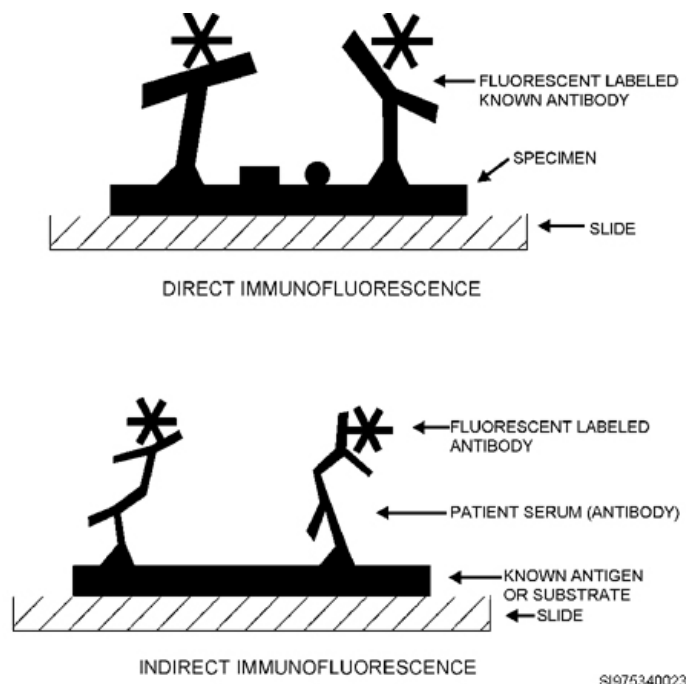


Figure 2-8. Example of direct and indirect immunofluorescence.

#### *Fluorescence polarization immunoassays*

Fluorescence polarization immunoassays (FPIA) are used primarily for the analysis of small molecular weight substances, such as drugs and hormones. The tracer in FPIA is the analyte (substance to be measured) conjugated to a fluorochrome. The analyte in the patient's sample competes with the fluorescent-labeled analyte for binding sites on the antibody. The detection of bound versus free tracer is based on the tumbling or rotation characteristics of the molecule in solution and their interaction on excitation with a plane polarized light. Polarized light is generated in a vertical direction. This light excites the molecules and produces fluorescence. Larger molecules (i.e., tracer bound to antibody) rotate more slowly than small molecules (i.e., free tracer). When a beam of polarized light is passed through a solution containing bound and free tracer, both will absorb light, but only antibody-bound tracer, because of its slow rotation, will emit light in the same vertical direction. Antibody-bound tracer thus causes an increase in polarized light being transmitted to the detector (i.e., increase in fluorescence polarization). Free tracer, because of its rapid rotation, will emit light in different directions resulting in less polarized light transmitted to the detector; hence, there is a decrease in fluorescence polarization. The higher the concentration of analyte in the patient sample, the fewer antibodies available for binding to the tracer, which means there is a higher amount of free tracer. In FPIA, the amount of analyte in the sample is inversely proportional to the amount of fluorescence polarization.

#### *Flow-cell cytometry*

Immunofluorescence can also be used to identify distinct antigens on live cells in suspension. An example is flow-cell cytometry. Live cells within a solution are stained with various fluorochromes. The flow-cell cytometer aspirates the solution and a fluorescent active cell sorter (FACS) separates the cells according to their intensity of fluorescences. This technique is used for isolating different cell populations that exhibit distinct surface antigens. This procedure is important when diagnosing and monitoring leukemic and AIDS patients (because it can identify CD4 and CD8 T cells).

### ***Chemiluminescence immunoassays***

Molecules can also absorb chemical energy and be excited to higher electronic levels. Once in these high electronic levels, the molecules behave as if they were excited by the absorption of light energy. In chemiluminescence immunoassays, light emission is produced by the excitation of molecules through a chemical reaction as opposed to photoluminescence, in which the excitation occurs because of light absorption. Most of these assays utilized synthetic chemiluminescent compounds (luminol, luminol derivatives, or acridinium ester derivatives) as labels for antigens or antibodies. Photon emission is measured by a luminometer or liquid scintillation counter. Chemiluminescence has been used as an analytic technique to measure a number of substances. Although the low efficiency of photon emission limits its sensitivity, the stability of the reagents and the low background activity are advantages of these assays. Chemiluminescence energy transfer has been used to increase the sensitivity. When this reaction requires an enzyme catalyst, it is called bioluminescence.

### ***Immunohistochemical staining***

Immunofluorescence can be applied to histochemical and cytochemical techniques to detect antigens associated with tissues and cells. Polyclonal or monoclonal antibodies labeled with a fluorescent dye were first used as immunohistochemical stains. These techniques utilize the natural specificity of an antibody to identify and locate a variety of antigens, including immunoglobulins, cell surface markers, hormones, enzymes, oncofetal antigens, viral/bacterial substances, etc., many of which were previously undetectable by cytochemical staining. Unfortunately, immunofluorescent staining is of limited sensitivity and requires a frozen tissue section. In addition, a fluorescent microscope is required for reading the slides.

### ***Immunoperoxidase staining***

Antibodies may be conjugated to other markers such as enzymes. Horseradish peroxidase, alkaline phosphatase, and avidin-biotin, are enzyme-conjugated labels that are utilized as visual tags to detect the presence of antibodies. Immunoperoxidase techniques, utilizing monoclonal or polyclonal antibodies, are many times more sensitive than immunofluorescent methods. They can usually be performed on fixed and paraffin embedded tissue. Immunoperoxidase-stained sections can be examined with an ordinary light microscope and are able to withstand long storage. The introduction of immunochemical techniques has brought a new era to pathological diagnosis.

### ***Monoclonal antibodies***

Monoclonal antibodies are pure populations of a specific immunoglobulin class that have an identical structure and combine with a single specific antigen. They are derived from a single clone of B lymphoid cells, usually from either malignant secretory B cells or a hybridoma.

### ***Polyclonal antibodies***

Polyclonal antibodies are different classes of immunoglobulins that are generally prepared by immunizing animals with human lymphocytes. If cells from the thymus are used for the preparation, the serum used is antithymocyte serum (ATS). Often, this serum is further fractionated to obtain the globulin portion termed antithymocyte globulin (ATG). Other antibodies are prepared from thoracic duct lymphocytes, splenic cells, or peripheral blood lymphocytes obtained by leukapheresis. These are referred to as either antilymphocyte serum (ALS) or antilymphocyte globulin (ALG).

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 209. Basic procedures

1. Match the terms in column B with the statement in column A. Each element in column B may be used once, more than once, or not at all.

#### Column A

- \_\_\_\_(1) Is used to complete the antibody bridging and increase lattice formation.
- \_\_\_\_(2) Utilizing light scatter to measure antigen-antibody complexes.
- \_\_\_\_(3) No hemolysis = positive test; Hemolysis = negative test.
- \_\_\_\_(4) Reactants include a hapten (ligand) linked to an enzyme, antibody directed against the hapten, and a substrate.
- \_\_\_\_(5) Direct or indirect agglutination procedure using erythrocytes.
- \_\_\_\_(6) Antigen and antibody placed opposite of each other and gel plate is placed in an electrical field.
- \_\_\_\_(7) Derived from a single clone of B lymphoid cells.
- \_\_\_\_(8) Usually uses a gamma emitter as a tracer.
- \_\_\_\_(9) Using antigens in their native state.
- \_\_\_\_(10) Prepared by immunizing animals with human lymphocytes.
- \_\_\_\_(11) Placing an antigen solution on top of an antibody solution.
- \_\_\_\_(12) A general term used for heterogeneous enzyme immunoassays.
- \_\_\_\_(13) Antibody added to a gel, antigen added to a well and electrophoresed resulting in a tapered pattern.
- \_\_\_\_(14) Fluorescein labeled antibody reacts with bacteria, spirochetes, or viruses impregnated on a slide.
- \_\_\_\_(15) No agglutination = positive test; Agglutination = negative test.
- \_\_\_\_(16) Used primarily for the analysis of small molecular weight substances, such as drugs and hormones.
- \_\_\_\_(17) Antibody is added to a liquefied gel, antigen added to cut out wells.
- \_\_\_\_(18) In the second phase, fluorescent anti-human globulin is added to the slide.
- \_\_\_\_(19) Agglutination procedures utilizing particles as passive carriers.
- \_\_\_\_(20) Antigen placed in a well, electrophoresed, and then an anti-human antibody is added.

#### Column B

- a. Direct agglutination.
- b. Indirect agglutination.
- c. Hemagglutination.
- d. Agglutination inhibition.
- e. Anti-human globulin.
- f. Fluid phase immunodiffusion.
- g. Gel phase immunodiffusion.
- h. Ouchterlony double diffusion method.
- i. Single radial immunodiffusion.
- j. Countercurrent immunoelectrophoresis.
- k. Rocket immunoelectrophoresis.
- l. Immunoelectrophoresis.
- m. Turbidimetry and nephelometry.
- n. Complement fixation.
- o. RIA.
- o. ELISA.
- p. EMIT.
- q. Direct fluorescent antibody technique.
- r. Indirect fluorescent antibody technique.
- s. FPIA.
- t. Monoclonal antibodies.
- u. Polyclonal antibodies.

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## Answers to Self-Test Questions

### 206

1. In dried blood or serum, HBV can be stable at room temperature for up to 7 days. HIV can be infectious for up to 3 days at room temperature and longer if in an aqueous environment.
2. The factors determining infectivity of HBV and HIV include; (1) concentration of virus, (2) duration of contact, (3) presence of skin lesions or abrasions on health-care worker, and (4) the immune status of the health care worker from HBV vaccination.
3. These viruses can directly enter the body by percutaneous inoculation, contamination of skin, exposure of mucus membranes, and through aerosols. Indirect entry can be through contaminated work surfaces or equipment.
4. Most antigens and antibodies are highly sensitive to changes in temperature, oxidation, and proteolytic degradation.
5. Split the specimen into different tubes if repeat or confirmatory testing is probable or required.
6. Analytic QC and nonanalytic control.
7. Antigen and antibody interaction.

### 207

1. During the acute phase of the illness and again during the convalescent period.
2. A central theory of serological testing is the manifestation of a rise in titer from the acute specimen to the convalescent specimen.
3. Dilutions; allowing the dilute specimens to react with a constant volume of antigen.
4. In a mathematical progression resulting in a dilution which is inversely proportional to the concentration of the substance being diluted.
5. Each dilution reduces the concentration of the specimen by one half.
6. A titer expresses the units or parts present in a total volume and is written as "a titer of 1:2 or 1:10."
7. The basic components are a Plexiglas or plastic sheet (drilled with a series of U-shaped or V-shaped wells) and calibrated droppers, loops, or pipettes.
8. This is a very rapid technique and cuts down the time and equipment required to perform large numbers of specimen dilutions.
9. For most infectious diseases, an increase in the person's titer of two doubling dilutions is considered diagnostic of a current infection; four fold rise in titer.
10. The type of cells selected depends on the type of antigen they carry and the type of antibody to isolate or quantify.

### 208

1. (1) i.  
(2) d.  
(3) h.  
(4) n.  
(5) f.  
(6) k.  
(7) b.  
(8) j.  
(9) c.  
(10) g.  
(11) b.  
(12) l.  
(13) a.  
(14) j.

(15) e.

(16) m.

**209**

1. (1) e.

(2) m.

(3) n

(4) q.

(5) c.

(6) j.

(7) u.

(8) o.

(9) a.

(10) v.

(11) f.

(12) p.

(13) k.

(14) r.

(15) d.

(16) t.

(17) i.

(18) s.

(19) b.

(20) l.

**Do the unit review exercises before going to the next unit.**

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### Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

18. (206) Which one of the following is *not* a factor to the laboratory technician for determining infectivity of the human immunodeficiency virus and the hepatitis B virus?
- a. Duration of contact.
  - b. Concentration of virus.
  - c. Immune status of the patient from HBV vaccination.
  - d. Presence of skin lesions or abrasions on health-care worker.
19. (206) The human immunodeficiency virus and the hepatitis B virus can *indirectly* enter the body through
- a. contamination of skin.
  - b. percutaneous inoculation.
  - c. exposure of mucus membranes.
  - d. contaminated work surfaces or equipment.

- 
- 
20. (206) Which of the following is *not* a factor to consider when checking the adequacy of the specimen collected for immunology testing?
- The presence of hemolysis or lipemia.
  - What medications the person is taking.
  - Know the sample type recommended by the manufacturer of the test kit.
  - The timing of acute and convalescent specimens collected during an infectious process.
21. (206) Quality control in immunology is *only* unique or different from the other sections of the laboratory in the area of
- temperature recording.
  - lot-to-lot correlations of test kits.
  - antigen and antibody interaction.
  - equipment and instrument maintenance.
22. (207) A high titer indicates that the patient
- has a severe infection.
  - has a high degree of immunity.
  - can mount a great immune response.
  - has a high amount of antibody present in the specimen.
23. (207) A four-fold rise in titer, from an acute to a convalescent sample, indicates
- an immunity.
  - a past infection.
  - a severe infection.
  - a current infection.
24. (208) Which of the following reactions is the result of an insoluble antigen with an antibody?
- Precipitation.
  - Agglutination.
  - Ligand assays.
  - Complement activation.
25. (208) Prozone is an excess in
- antigen concentration; resulting in lattice formation.
  - antibody concentration; resulting in lattice formation.
  - antigen concentration; resulting in no lattice formation.
  - antibody concentration; resulting in no lattice formation.
26. (208) What method may be considered the *first* labeling technique?
- RIA.
  - EIA.
  - Precipitation.
  - Agglutination.
27. (208) The label allows all reactions to be
- measured through light scattering and increases its sensitivity.
  - measured, amplifies the power of the test, and increases its sensitivity.
  - measured through liquid scintillation counters and amplifies the power of the test.
  - measured macroscopically, amplifies the power of the test, and increases its sensitivity.
28. (208) In ligand assays the binding reagent can be a
- bacteria, virus, or antigen.
  - receptor, virus, or antibody.
  - bacteria, protein, or antigen.
  - receptor, protein, or antibody.

29. (208) Which of the following assays require a separation of the free label from the bound label before quantitating the bound label?
- Ligand assays.
  - Labeled assays.
  - Homogenous assays.
  - Heterogeneous assays.
30. (208) What type of measurement generates a spectrum of signal responses that correlates with the concentration of the analyte of interest?
- Qualitative.
  - Quantitative.
  - Semi-qualitative.
  - Semi-quantitative.
31. (209) What procedures use antigens in their native state and are found naturally on the surfaces of bacteria, fungi, or human cells?
- Hemagglutination.
  - Direct agglutination.
  - Agglutination inhibition.
  - Indirect or passive agglutination.
32. (209) Which one of the following terms is used when both the antigen and antibody diffuse through the medium?
- Immunodiffusion.
  - Immunoelectrophoresis.
  - Double immunodiffusion.
  - Single radial immunodiffusion.
33. (209) Which immunodiffusion methods add antigens and antibodies to opposite wells and place the gel plate in an electrical field?
- Rocket immunoelectrophoresis.
  - Single radial immunodiffusion.
  - Immunofixation electrophoresis.
  - Countercurrent immunoelectrophoresis.
34. (209) A positive complement fixation reaction is indicated by
- hemolysis.
  - precipitation.
  - agglutination.
  - no hemolysis.
35. (209) A fluorescence immunoassays uses
- an enzyme label.
  - a fluorochrome label.
  - a radioactive isotope label.
  - a light-emitting chemical label.
36. (209) Which one of the following methods *usually* uses the gamma emitter  $^{125}\text{I}$  as the tracer?
- EIA.
  - RIA.
  - FPIA.
  - ELISA.



37. (209) Which one of the following enzyme immunoassay procedures is considered homogeneous?
- a. IFA.
  - b. FPIA.
  - c. EMIT.
  - d. ELISA.
38. (209) Which one of the following procedures uses unlabeled patient's serum to react with a known antigen in the first phase and a fluorescent-labeled anti-human globulin in the second phase?
- a. Flow-cell cytometry.
  - b. Direct fluorescent antibody technique.
  - c. Indirect fluorescent antibody technique.
  - d. Fluorescence polarization immunoassays.
39. (209) Which one of the following is derived from a single clone of B lymphoid cells, usually from either malignant secretory B cells or a hybridoma?
- a. Polyclonal antigen.
  - b. Monoclonal antigen.
  - c. Polyclonal antibody.
  - d. Monoclonal antibody.

**Please read the unit menu for unit 3 and continue ➔**

## **Student Notes**

## Unit 3. Immunology Testing

<b>3-1. Routine Testing.....</b>	<b>3-1</b>
210. Routine tests performed.....	3-1
211. Syphilis testing .....	3-11
<b>3-2. Viral testing.....</b>	<b>3-18</b>
212. Hepatitis testing .....	3-18
213. Human Immunodeficiency Virus testing .....	3-25
214. Miscellaneous viruses .....	3-27

**I**MMUNOLOGY TESTING applies the techniques, methodologies, and procedures, (presented in unit 2), for diagnosis of specific disorders and diseases. Unlike other sections in the laboratory that test for specific chemicals, blood cells, or bacteria, immunology procedures are used to detect numerous substances (e.g., bacteria, spirochetes, viruses, and other immune complexes). These test range from simple agglutination screening procedures to multi-step confirmatory test. Without further delay, let's begin with routine testing.

### 3-1. Routine Testing

I am not sure that routine testing is the appropriate term to describe the following procedures. However, I do believe that these procedures are probably the most common immunology test performed within all Air Force laboratories. Although routine, an undiagnosed case of any of these disorders could result in the loss of countless duty hours, cause needless suffering, or even take the life of the patient.

#### 210. Routine tests performed

This section will discuss the “routine” test performed in immunology:

- Cold agglutinin.
- Infectious mononucleosis.
- Streptococcal antibodies.
- Rheumatic diseases.
- Pregnancy testing.

The test for cold agglutinins is the same technique practiced for decades; it's simple, yet very effective.

#### **Cold agglutinins**

Cold agglutinins are autoantibodies that agglutinate human red blood cells at temperatures <37°C. They are IgM antibodies with a specificity for the Ii system. Approximately 95% of all cold agglutinins react with anti-I; the other 5% react with anti-i or anti-Pr. Cold agglutinins are present in different illnesses and are divided into 3 categories: (1) primary cold agglutinin disease, (2) secondary cold agglutinin disease, and (3) paroxysmal cold hemoglobinuria.

#### *Clinical significance*

Cold agglutinins can cause hemolysis through physical agglutination or by complement activation.

#### *Primary cold agglutinin disease*

This disease is marked by periods of painful acrocyanosis (uneven blue or red discoloration of the skin on the extremities). Acrocyanosis is due to the agglutination of red blood cells in the peripheral

Circulation upon exposure to the cold. This disease is usually seen in the elderly and is managed by avoiding cold environments. Chronic lymphoproliferative disorders must be ruled out before a final diagnosis of primary cold agglutinin disease is made.

#### *Secondary cold agglutinin disease*

Secondary cold agglutinin disease is associated with a variety of infections; mycoplasma pneumonia and infectious mononucleosis are the most common. Increased cold agglutinin titers are used as helpful test for the presumptive diagnosis of mycoplasma pneumonia. In infectious mononucleosis patients, cold agglutinins are directed against the “i antigen” and requires fetal red blood cells for detection. Secondary cold agglutinin disease is also associated with a variety of lymphoproliferative disorders. Before diagnosing secondary cold agglutinin disease, all positive cold agglutinin tests should be investigated to rule out lymphoproliferative disorders.

#### *Paroxysmal cold hemoglobinuria*

This is a rare disease, which many are transient or chronic. It constitutes 10% of the cold autoimmune hemolytic anemias. Paroxysmal cold hemoglobinuria may be seen as a primary disease or secondary to syphilis or viral infections (most often with measles, mumps, or infectious mononucleosis). This is an IgG autoantibody that binds to P<sub>1</sub> and P<sub>2</sub> antigens, on human erythrocytes, at temperatures below 20°C. This autoantibody can also activate complement. Hemolysis occurs only after the blood has been chilled and the sensitized cells are warmed to 37°C. A positive direct antiglobulin test (DAT) can only be seen at temperatures below 24°C. Acute attacks usually resolve spontaneously, but occasionally a transfusion is required.

#### **Laboratory identification**

Blood collected for cold agglutinin testing requires special care. If improperly handled, the cold agglutinins can be absorbed by the patient's erythrocytes and tied up in the clot as the specimen cools. Equipment used to draw blood for cold agglutinins must be pre-warmed to 37°C by holding the syringe or air-evacuated tube tightly in your hand for a couple of minutes. Once the specimen is obtained, it must be kept warm and transported immediately to the laboratory where it can then be placed immediately into a 37°C waterbath or heat block. Allow a full 30 minutes to assure complete clotting, immediately centrifuge, and quickly separate serum from cells (the serum must be cell free). Although testing should take place immediately, the serum can be frozen until testing. A 5% suspension of washed human group O cells serves as the antigen in this test. Patient serum is serially diluted with saline. One drop of antigen is added to each dilution, and the test is incubated at 2 to 4°C overnight. However, a preliminary reading can be obtained in 1 hour. Upon removing the tubes from the refrigerator, read them immediately for agglutination and record the titer, then allow the tubes to warm, re-centrifuge, and re-read. True cold agglutinins will disperse upon warming. Titers of 1:16 or less are considered insignificant. Titers of  $\geq 1:32$ , if accompanied by a typical clinical picture, are significant. **NOTE:** A known positive cold agglutinin control should be set up with every batch of test samples.

#### **Infectious mononucleosis**

Infectious mononucleosis (IM) is the most common illness caused by the Epstein-Barr virus (EBV) which is a member of the herpes virus family. EBV is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma, lymphomas, HIV-related conditions, and has been proposed as the cause of chronic fatigue syndrome. EBV transmission is almost exclusively through salivary contact. It infects the oropharyngeal epithelia where it is shed in the saliva and then spreads to the subjacent B cells. Cytotoxic T cells are activated and results in the appearance of atypical lymphocytes in the peripheral blood. Refer back to unit 1 and figure 1-9 for a review. B cell proliferation occurs with the production of plasma cells that produce heterophil antibodies.

### ***Clinical significance***

IM is a disease frequently tested for in the clinical immunology section. It is an acute or subacute benign disease. Young adult's ages 16 to 25 are those most frequently affected. Consequently, the "kissing disease" is a significant problem for the military services, especially training centers. By the age of 30, the presence of EBV antibodies approaches 90% in humans worldwide. The incubation period varies from 4 to 7 weeks. IM is characterized by fever, headache, and sore throat with an exudate, enlarged lymph nodes, splenomegaly, malaise, and hepatitis. The acute disease usually persists for 2 to 4 weeks and is followed by a long convalescence that can last for months. Complications of IM include hemolytic anemia, aplastic anemia, encephalitis, Guillain-Barré syndrome, myocarditis, nephritis, and hepatic failure. A few patients will become jaundice and may develop a macular rash. The infection results in a lifelong immunity, but, the individual is infected and a carrier for life. Reactivation of the EBV is possible in the immunocompromised or immunosuppressed host. There is a group of illnesses that have overlapping clinical and serological findings, they are known as *IM-like illnesses*. IM-like illnesses (without heterophil antibodies) can be caused by EBV, HIV, cytomegalovirus (CMV), the newly described human herpes virus-6 (HHV-6), *Toxoplasma gondii*, and, rarely, by certain drugs. As you can see a correct diagnosis is very important.

### ***Laboratory identification***

The organism reproduces in the B lymphocytes of the infected individual but cannot be recovered from circulating blood. However, the EBV can be recovered from throat washings and saliva of those infected. Recently, it has been recovered from both semen and cervical epithelium, but it is not known if it is sexually transmitted. IM is usually diagnosed through serological tests that detect the presence of heterophil antibodies in serum. Heterophil antibodies are stimulated by one antigen but react with completely different antigens. Examples of heterophil antibodies are IM antibodies that react with sheep, beef, and horse erythrocytes, nontreponemal antibodies that react to reagin (i.e., syphilis), and nonimmune antibodies that react to ABO blood groups. The IM antibody is one of the three heterophil antibodies that display the ability to cross-react with sheep, beef, and horse erythrocytes. Serum sickness and Forssman antibodies are the other two. Serum sickness antibodies are produced from exposure to serum from different animal species. They also react with sheep, beef, and horse erythrocytes. Forssman antibodies are produced in response to certain bacteria, but they only react with sheep and horse erythrocytes. These antibodies must be absorbed out of the serum, so they do interfere with IM testing.

### ***Paul-Bunnell screening test***

Paul-Bunnell is a hemagglutination procedure for detecting heterophil antibodies. In test tubes, inactivated patient serum is serially diluted with normal physiologic saline. Add a specific amount of a 2% suspension of washed sheep cells (in saline) to each tube. Incubate tubes for 1 hour at 37°C or overnight at room temperature. After incubation, centrifuge tubes and examine for macroscopic agglutination. A titer of >1:56 indicates the presence of heterophil antibodies. This is a qualitative test and doesn't identify which heterophil antibody is present.

### ***Davidsohn differential test***

In 1929, Dr. Davidsohn developed a test to differentiate the three common types of heterophil antibodies by incorporating an absorption step. Therefore, by comparing the agglutinins remaining after absorption with guinea pig kidney (GPK) cells and beef erythrocytes you can identify the heterophil antibody present. Add inactivated patient serum to a tube with a 20% suspension of GPK cells and a tube of a 20% suspension of beef erythrocytes. After a 5 to 10 minute incubation, centrifuge tubes, and remove the supernatant. Serially dilute the supernatant and add a 2% cell suspension of sheep erythrocytes. Incubate at room temperature for 2 hours. Gently shake and examine for macroscopic agglutination. This test is specific, but time consuming. The agglutination patterns are listed in the following table.

Heterophil Antibody	Absorbed by GPK Cells	Absorbed by Beef Erythrocytes
IM	No	Yes
Serum sickness	Yes	Yes
Forssman	Yes	No

#### *Rapid slide tests*

A number of slide modifications of the basic GPK cells and beef erythrocytes absorption tests are available commercially. Since absorption tests seem to be the most widely accepted method for the differential identification of the heterophil antibodies, these slide tests offer a distinct advantage over the original tube test—speed. In addition, these tests delete the need for a presumptive test. Later, horse erythrocytes were found to be more sensitive, with fewer false positives than sheep erythrocytes, so they were used. The reagents can be lyophilized (dried) on a card, or in a liquid form. The serum is added to a card with dried GPK cells, beef erythrocytes stoma, and horse erythrocytes (liquid reagents are added to a glass slide). Mix serum and reagents in the order described by the OI or manufacturers instructions in the product insert. Incubate as indicated by the OI and examine for macroscopic agglutination. It is important to follow the OI or manufacturers' instructions exactly for correct interpretation of results. Although some of these manufacturers include procedures for diluting the antibodies, it is not recommend because the titer does not parallel with the severity of the disease. Liver function tests are used for following the course of the disease.

#### *EIA EBV tests*

Recently a 4 to 10 minute heterophil EIA test has been developed. The EIA tests have greater sensitivity, longer shelf life, and more distinct visual endpoints, but are more expensive than agglutination procedures. Hence, they haven't been extensively evaluated. EIA procedures incorporate sheep or bovine erythrocytes on a solid-phase support (i.e., plastic tube, disks, or membranes). Incubate serum with the solid-phase support. If IgM specific heterophil antibodies are present, they will bind with the antigens attached to the solid support. After washing, add an enzyme-labeled anti-human IgM, incubate, wash, and add the appropriate substrate. After a short incubation, measure the colormetric endpoint to determine the heterophil test results.

#### *EBV specific test*

The antibodies present in IM are heterophil and EBV specific antibodies directed against the viral capsid antigen (VCA), early antigen (EA), and Epstein-Barr nuclear antigen (EBNA). The table below describes the course of these specific antibodies in IM and other EBV-associated diseases.

EBV Antibody	Appearance Time	Detection Time
IgM Anti-VCA	Onset of disease	12 weeks after onset
IgG Anti-VCA	Onset of disease	Life-long
Anti-EA	Onset of disease	8 to 12 weeks after onset
Anti-EBNA	2 to 3 months after onset	Indefinite

#### *Indirect fluorescent antibody procedures*

Indirect fluorescent antibody (IFA) procedures can detect the antibodies produced against the Epstein-Barr virus. This procedure can identify the stage of the disease, which results in better disease management of EBV-associated diseases. Other procedures used for detecting EBV antibodies include complement fixation (CF) and hemagglutination inhibition (HAI). **NOTE:** Known positive and negative controls are run with each batch of patient samples for all procedures mentioned above.

#### **Streptococcal antibodies**

The genus *Streptococcus* is a diverse group of gram-positive cocci, which cause severe acute infections and complications after infection. Most streptococcal infections don't produce immunity unless the microorganism produces toxins (e.g., *Streptococcus pyogenes* exotoxins associated with scarlet fever).

### ***Classifications***

Lancefield's serological classification system was based on antigenic material or the C carbohydrate on the bacteria cell wall. In the Lancefield system, the streptococci are placed in groups A through O based on their antigenic make-up: all group A streptococci are antigenically identical for this carbohydrate, group B the same, and so on. Group A streptococci are further divided into serotypes by the proteins in their cell wall: M, T, and R. M protein is one of the virulence factors produced by *S. pyogenes* and there are approximately 80 antigenically distinct group A streptococci with M serotypes. These M serotypes have a significant disease association. The M proteins do produce protective IgG antibodies; however, there are so many different serotypes of the M protein that reinfection with another strain is common.

### ***Clinical significance***

Different group A serotypes are involved in skin infections (impetigo and cellulitis) than those involved in upper respiratory infections (e.g., pharyngitis). Not all infections with *S. pyogenes* lead to complications, but it is known that streptococcal pharyngitis leads to rheumatic fever and, both, skin infections and streptococcal pharyngitis lead to acute glomerulonephritis.

#### ***Rheumatic fever (RF)***

Rheumatic fever usually occurs 3 to 4 weeks after the infection and is seen in about 2 to 3% of untreated cases of streptococcal pharyngitis. This nonsuppurative infection involves the heart (rheumatic carditis), central nervous system (Sydenham chorea), or joints (rheumatic polyarthrititis). Rheumatic carditis may become chronic and progressively damage the valves of the heart. Patients with a history of rheumatic carditis are at a significantly higher risk of developing cardiac malfunctions and endocarditis at a later date. The other sites of infections rarely result in permanent damage. The exact process that leads to RF is not completely understood. However, there are several theories of this process.

1. Direct damage or destruction of the tissue results from the streptococcal organism or the enzymes produced.
2. A serum sickness-like reaction is mediated by the antigen-antibody complexes.
3. An autoimmune response is induced by the infection. This is the most popular theory, because group A streptococci share epitopes with some host cells, tissues, and organs. Also, antibodies directed against heart tissue have been identified in some patients with rheumatic carditis.

#### ***Acute glomerulonephritis***

Acute glomerulonephritis (AGN) is also a nonsuppurative infection and is a complication from streptococcal pharyngitis or streptococcal skin infections. Its occurrence varies from 0.03 to 18% and begins around 10 days after pharyngitis and 18 to 21 days after a skin infection. The symptoms of AGN are proteinuria, hematuria, hypertension, and edema. The exact disease process is also unknown. As with RF, there are many theories. The most popular theory is that the circulating antigen-antibody complexes deposit in the glomerular basement membrane, then activate complement, which damages the glomeruli. Complement-mediated lysosome is released from the WBCs, platelets aggregate in the area, and there is a build-up of fibrin (threads) and fibrinogen. These mechanisms result in capillary obstruction, which leads to impaired renal function. Progressive, irreversible loss of renal function has been reported in adults.

### ***Laboratory identification***

Because the streptococcal organisms can't be isolated from the organs themselves, the only other means of demonstrating a recent infection is serological. Streptococci group A, B, C, F, and G produce extracellular products (proteins and enzymes) that may evoke protective immunity. Streptolysin O and S are cytopathic proteins (toxins) that inhibit phagocytosis and killing by leukocytes. A variety of proteinases exist (e.g., streptokinase, hyaluronidase, and deoxyribonuclease)

and they enhance the pathogenicity of the organism. During infection, antibodies may be produced to all these factors: antistreptolysin O, antistreptokinase, antihyaluronidase, and antideoxyribonuclease-B.

#### *ASO neutralization test*

Streptolysin O and S, produced by many strains of beta-hemolytic streptococci (especially group A), are responsible for the hemolysis observed on blood agar culture plates. Antistreptolysin O neutralizes this hemolytic activity. This procedure uses the hemagglutination inhibition methodology. In the ASO neutralization procedure, a dilution of patient serum is incubated with a standardized streptolysin O reagent. (These dilutions usually start with a 1:10 and are diluted in set manner.) After incubation, rabbit or washed human group O red blood cells are added to each dilution tube. The tubes are incubated for 30 minutes at 37°C in a water bath or heat block. Then the tubes are centrifuged and examined for hemolysis. If the patient has developed antistreptolysin O antibodies, the standardized streptolysin O reagent will be inhibited or neutralized, and the red blood cells will not be hemolyzed. If no antibodies are present, the red blood cells will be hemolyzed by the free streptolysin O reagent. Quality control includes a tube with only red blood cells (no hemolysis seen) and a tube with buffer, streptolysin O reagent, and red blood cells (hemolysis seen). If the quality control tubes are correct, the last dilution showing no hemolysis is the titer of the test.

Test results are reported as Todd units (international units) which is the reciprocal of the original serum dilution before the addition of red blood cells. For example, if tube 5 exhibits no hemolysis and it contains 0.6 ml of serum diluted 100 times, then the reciprocal of 0.6 is 10/6. This is multiplied by 100 (the dilution) for a result of 166 Todd units ( $10 \div 6 = 1.66$ ;  $1.66 \times 100 = 166$ ). Acute and convalescent samples should always be tested because of the wide range of normal values. It is important to demonstrate a fourfold increase in titer for diagnosis. Normal results for children <5 years of age is < or = to 333 Todd units and for adults, < or = 250 Todd units. This difference accounts for the higher incidence of beta-hemolytic streptococcal infections in young children. Persistent low titers suggest a past infection versus a recent infection with streptococci.

#### *Rapid ASO procedure*

In the rapid latex agglutination ASO procedure, latex particles are coated with streptolysin O and mixed with patient serum. If antistreptolysin O is present, visible agglutination will be seen. Visible agglutination is a positive result and no agglutination is a negative result.

#### *Antihyaluronidase test (AHT)*

The numbers of patients that develop antibodies to hyaluronidase are somewhat lower than those who develop antibodies to streptolysin O. However, this procedure will detect antibodies produced from either pharyngitis and skin infections, whereas ASO only detects antibodies from pharyngitis. This is also an inhibition or neutralization procedure. If the antibody is present it neutralizes the enzyme hyaluronidase and prevents the enzymatic break down of the substrate potassium hyaluronate.

#### *Antideoxyribonuclease-B B test (DNase B)*

*Streptococcus pyogenes* produces four DNases: A, B, C, and D. Almost all strains of group A streptococci produces DNase B. This test is thought to be the single most reliable test to detect previous group A streptococcal infections. This is also a neutralization procedure; anti-DNase B (streptodornase) in the patient's serum prevents DNase B from hydrolyzing DNA.

#### *Streptozyme screening test*

The Streptozyme is a 2-minute slide hemagglutination procedure, which semi-quantitatively measures multiple antibodies to streptococcal extracellular products. The reagent consists of sheep erythrocytes coated with streptococcal enzymes. The sheep erythrocytes are sensitized simultaneously with streptolysin O, streptokinase, hyaluronidase, deoxyribonuclease B (DNase B), and nicotinamide adenine dinucleotides (NADase). Agglutination of the erythrocytes indicates the presence of



antibodies in the serum against one-or-more of the streptococcal enzymes. Test results can be considered acceptable if the supplied control sera give the expected results according to the manufacturer's instructions.

### ***Review of test***

All procedures should be performed on paired acute and convalescent serum. A fourfold rise in titer is diagnostic. The conversion of titer results to Todd units is the same for each procedure. Positive and negative controls should be performed with each procedure. Specimens should be free of hemolysis, lipemia, and bacteria because of interference. The table below reviews the procedure and its relative reliability for preceding group A streptococcal infections.

Test	Titer Peaks	RF	AGN
ASO	3 to 6 weeks after infection	+++	0
AHT	3 to 6 weeks after infection	++	++
DNase B	4 to 6 weeks after infection, remains for months	++++	++++
Streptozyme	3 to 6 weeks after infection	+	+
As you can see from this table, a combination of an ASO and DNase B will provide the best diagnostic results.			

### **Rheumatic diseases**

Most of the rheumatologic disorders are autoimmune in nature. Nonetheless, the exact processes of these diseases are not completely understood. We do know that autoimmune diseases are characterized by the production of antibodies that react with self antigens. Some self-reactivity is normal; for example, interactions between lymphocytes and endothelial cells, and, also, between T cells and antigen presenting cells. However, we are more concerned with abnormal self-reactivity that results in pathology. An example of abnormal autoimmune activity is the production of autoantibodies in rheumatic fever against cardiac tissue. In addition to whether the response is normal or abnormal, it is important for the physician to know what evoked the autoimmune response—a foreign invader or self antigen. Many studies are devoted to foreign antigens that cross-react with self antigenic determinants and possibly elicit the production of autoantibodies.

### ***Effector mechanisms in rheumatic diseases***

The effector mechanisms present in immunity and allergies (i.e., antigen-antibody complex mediated, cell mediated, cytotoxic, and anaphylactic mechanisms) are also present in autoimmune responses. One effector mechanism may seem to dominate, but, in fact, there is probably more than one effector mechanism involved in the disease. Autoimmune diseases usually affect a single type of tissue or organ system, but, some affect more than one tissue type or organ. Rheumatic diseases include rheumatoid arthritis (RA), juvenile arthritis, systemic lupus erythematosus (SLE), Sjögren's syndrome, progressive systemic sclerosis, polymyositis-dermatomyositis, Behçet's disease, ankylosing spondylitis, Reiter's syndrome, psoriatic arthritis, relapsing polychondritis, relapsing panniculitis, hereditary complement deficiency (collagen vascular diseases), and hypogammaglobulinemia with arthritis. Our study is limited to rheumatoid arthritis and systemic lupus erythematosus.

### ***Rheumatoid arthritis***

Rheumatoid arthritis (RA) is a chronic, recurrent systemic inflammatory disease primarily involving the joints. It affects 1 to 3% of the population worldwide with a female to male ratio of 3:1. There is no evidence of racial, geographical, or climatic predisposition. There is growing evidence that indicates that a genetic predisposition exists. Studies reveal that approximately 70% of RA patients have the HLA-DR4 haplotype. There is also an increased incidence of RA in first-degree relatives of RA patient's. RA is primarily diagnosed by clinical features established by the American Rheumatism Association or by using the alternative New York classification.

### *The hypothetical immunological mechanism of RA*

The cause of the unusual immune response and the following inflammation of RA are still unknown. It is theorized that an antigenic stimulus activates the synovial B lymphocytes to produce an IgG that is recognized as foreign. This stimulates an immune response within the joint that produces a different IgG, monomeric IgM, and pentameric IgM immunoglobulins. These are known as rheumatoid factors (RF). The IgG (rheumatoid factor complexes or aggregates of IgG) activate the complement system via the classic activation pathway. When complement products breakdown, they accumulate in the joint and stimulate the alternative activation pathway of complement. If you recall from unit 1, complement is a potent mediator of inflammation. Histamine is released, chemotaxins induce the direct migration of neutrophils and monocytes, and membrane damage with cell lysis occurs with the activation of complement. Various chemicals including prostaglandins, leukotrienes, lysosomes, enzymes, proteases, collagenases, and cytokines released by the different cells amplify the inflammatory responses causing damage to the cartilage and surrounding tissue.

### *Clinical significance*

Rheumatoid arthritis is a chronic inflammatory disease affecting the joints and synovial membranes that normally affects individuals between 20 and 40 years of age. The disorder usually progresses in 3 distinct stages. The first stage is the beginning of synovitis caused by the rheumatoid factors. Second, the subsequent immune responses (activation of complement, etc.) amplify the initial inflammatory reaction. And, third, a transition from an inflammatory reaction in the synovium to a proliferative destructive process that produces tissue damage within the joint (articular) and outside the joint (extra-articular). Articular symptoms include stiffness and joint pain that is usually worse in the morning, inflammation, swelling, warmth, erythema (redness of skin), and tenderness. Inflammation generally starts in the small joints of the hands and feet. Later in the course of the disease, it moves to the larger joints (knees, hips, shoulders, etc.). For certain patients large-joint involvement predominates. The cervical spine may also be affected. Joint inflammation leads to the weakening of the tendons, ligaments, and other support structures. The disease may ultimately progress to severe skeletal deformity and complete immobilization of the affected joints. Around 20 to 25% of the patients will have extra-articular complications. These include rheumatoid nodules that can affect the bones and/or organs, pleurisy, fibrosis of the lungs, cardiac disease, inflammation of the blood vessels, and inflammation of the eyes.

### *Laboratory identification*

Unfortunately, the mere presence of RF in serum does not confirm diagnosis of RA. Numerous other diseases may produce detectable levels of a rheumatoid factor in serum (e.g., SLE, Sjögren syndrome, syphilis, leprosy, tuberculosis, chronic active hepatitis, and active bacterial endocarditis). In addition, a negative test does not rule out RA. Some 25% of actual cases have been shown to produce negative tests. There are various tests and kits available to the laboratorian for assaying RF. Screening methods include latex fixation, latex agglutination, and hemagglutination. We speak of the test as a "latex fixation" because the antigen (gamma globulin) is fixed (absorbed) by the latex carrier particle. RA procedures are unique in that they use an immunoglobulin as the antigen to detect and react with RF (immunoglobulins). Newer techniques (RIA, ELISA, indirect immunofluorescence, and laser nephelometry) can detect all immunoglobulin classes of RF. Other techniques used to help diagnosis or follow the course of the disease include synovial fluid examinations, serum protein electrophoresis, cryoprecipitates, C-reactive protein, sedimentation rate, CBC, and antinuclear antibodies.

### *Systemic lupus erythematosus*

Systemic lupus erythematosus (SLE) is a chronic systemic inflammatory autoimmune disease that affects multiple organs. The American Rheumatism Association has also established 11 clinical and laboratory criteria for the diagnosis of SLE. However, only 4 of the 11 criteria must be seen simultaneously or serially during the time of observation. The exact cause of SLE is unknown. Possible etiologic agents are the effects of estrogen, genetic predisposition, extraneous factors, defects

in the immune system, or a combination of one or more of these factors. It has been well documented that certain drugs can induce a SLE-like syndrome. The disease follows a course of alternating exacerbations and remissions. It can be a mild disease confined to one or more organs or a fulminate fatal disease. It affects more females than males with a ratio of 4:1 and it is more common in nonwhites than in whites. The onset of the disease ranges from 2 to 90 years of age.

#### *Clinical significance*

Systemic lupus erythematosus doesn't follow a characteristic pattern; but the initial symptoms include fever, fatigue, and weight loss. One, more than one, or every organ maybe affected. Autoantibodies are produced and most of these antibodies fix complement and consequently damage the target tissue. Approximately 90% of the patients with SLE experience joint and muscle inflammation (polyarthralgia or arthritis). Some patients exhibit skin lesions or an erythematous rash that affects the areas of the skin exposed to ultraviolet light. A few patients develop the classic "butterfly" rash on the face. Renal disease and pleurisy are frequent and serious complications of SLE. Less common effects of SLE are heart disease, CNS involvement, vasculitis, gastrointestinal ulcerations, ocular involvement, and Sjögren syndrome.

#### *Laboratory identification*

Immunoglobulins of all classes may form antinuclear antibodies, but the anti-DNA antibodies are either IgG or IgM. Antinuclear antibodies (ANA) are immunoglobulins directed against antigens normally found in the nuclei of the cell. Go back to figure 1-1 of volume 1—*Hematology*, and review the schematic of a basic cell. This may help to understand antinuclear antibodies. These antigens are proteins, nucleic acid, or, most commonly, nucleoprotein complexes. ANAs are useful disease markers but have limited value in monitoring SLE, because ANAs are also found in healthy individuals. At this time, the preferred method for detecting ANAs is the indirect immunofluorescent assay. Human carcinoma cell lines (Hep2 or KB) are the antigens or substrates of choice. The typical immunofluorescence patterns seen are homogeneous, nucleolar, peripheral, and speckled. These patterns are associated with SLE and other rheumatic diseases. There are 3 major anti-DNA antibody patterns or types: (1) anti-single-stranded or "denatured" DNA (ss-DNA), (2) anti-double-stranded or "native" DNA (ds-DNA), and antibodies that react with both ss-DNA and ds-DNA. These antibodies can be quantitatively measured by RIA or ELISA techniques. High titers of anti-ds-DNA antibodies are essentially seen only in SLE. The hallmarks of active SLE are diminished serum complement and the presences of anti-ds-DNA.

#### **Pregnancy testing**

Fertilization is accomplished following successful completion of a complex sequence of events involving a spermatozoan and an egg. After fertilization is achieved and mitotic division is successfully initiated, it takes 6 days for the conceptus to travel from the fallopian tube to the uterus. Human chorionic gonadotrophin (hCG) is secreted by the embryonic tissues day 1 after implantation in the uterus. The rapid rise in hCG serum levels after conception makes it an excellent marker for early confirmation and, also, for monitoring pregnancy. It is a two-subunit glycopeptide hormone produced by the trophoblastic cells of the placenta. The hCG is also responsible for changing the ovary tissue of the menstrual cycle to the progesterone secreting tissue of pregnancy.

#### *Clinical significance*

Obviously, we know the clinical significance of pregnancy, but let's look a little deeper. In normal pregnancy, hCG levels change during gestation. The hCG level rises rapidly following conception and becomes detectable about the 21<sup>st</sup> day of gestation (the 1<sup>st</sup> day of the last menstrual cycle) or approximated 4 to 7 days after conception. hCG levels double approximately every 48 hours and peaks around 70 days after gestation. Then the levels decline slightly and plateau during the 2<sup>nd</sup> and 3<sup>rd</sup> trimester. hCG is also used to diagnose ectopic pregnancy and to evaluate threaten spontaneous abortion, trophoblastic tumors, testicular tumors, and some nontrophoblastic tumors. A sharply reduced or falling serum hCG level may indicate an abnormal pregnancy.

### ***Laboratory identification***

Before the 1960's (when agglutination immunoassays were developed), bioassays using frogs, rabbits, and mice were used to diagnose pregnancy. The first immunoassays included latex agglutination (LA), hemagglutination (HA), latex agglutination inhibition (LAI), and hemagglutination inhibition (HAI). Today, we use rapid agglutination inhibition (LAI or HAI) procedures performed on slides or in test tubes, EIA (e.g., antigen capture assays), and RIA procedures.

### ***Immunoassay specificity***

It should be noted that four glycoprotein hormones; thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone, and hCG; all have identical alpha ( $\alpha$ ) subunits but differ in their beta ( $\beta$ ) subunit. The specificity of all immunoassays depends on the specificity of the antibody used in that assay. In order for the assay to be specific for hCG, the antibody must recognize the unique section of the  $\beta$  subunit on the hCG molecule. In response to a specific antigen, the body will produce a polyclonal antibody response. This heterogeneous antibody response is due to the many cell lines involved. In order to detect the presence of a small portion of a given molecule (in this case the  $\beta$  subunit of the hCG molecule), an antibody of exact specificity has to be obtained. Monoclonal antibodies, against the  $\beta$  subunit of the hCG molecule, have been produced using a single clone of B lymphoid cells. These genetically engineered antibodies will not react with any substance other than human chorionic gonadotropin. The use of monoclonal antibody techniques has produced specific assays with little or no cross-reaction with the other glycoprotein hormones. The First International References Preparation of Human Chorionic Gonadotropin of Immunoassays (made available in 1975 from the World Health Organization) serves as the reference for hCG immunoassays.

### ***RIA and EIA assays***

You should already be familiar with agglutination and inhibition procedures, as well as RIA and EIA techniques, so we will not go over the exact procedures again. RIA and EIA quantitative hCG concentrations are expressed in mIU/ml, IU/ml, or ng/ml; where 1 mIU/ml is equal to 0.08 ng/ml. Routine pregnancy test (LAI and HAI) have a sensitive range from 75 to 1,500 mIU/ml, depending on the manufacturer and method. Membrane EIA procedures have a sensitivity range between 20 and 25 mIU/ml also depending on manufacturer. RIA procedures can be sensitive to a level of just 3 mIU/ml. In ectopic pregnancy, the hCG is less than that in the uterine pregnancy and is in the range of 150 to 800 mIU/ml. The weeks of gestation and the approximate normal hCG range is stated in the table below.

<b>Weeks of gestation</b>	<b>Approximate hCG normal range</b>
3 to 4	9 to 130
4 to 5	75 to 2,600
5 to 6	850 to 20,800
6 to 7	4,000 to 100,200
7 to 12	11,500 to 289,000
12 to 16	18,300 to 137,000
2 <sup>nd</sup> trimester	1,400 to 53,000
3 <sup>rd</sup> trimester	940 to 60,000

### ***High levels of hCG***

High levels of hCG may be associated with multiple pregnancies, eclampsia, polyhydramnios, erythroblastosis fetalis, testicular choriocarcinomas, seminomas, teratomas, and embryonal carcinomas. Trophoblastic tumors, such as hydatidiform mole and choriocarcinoma, secrete high levels of hCG in the range of 5,000 to 6,000,000 mIU/ml. To quantify these levels, it may be necessary to dilute the specimen sequentially for agglutination assays or use a quantitative RIA or

EIA method. When monitoring tumor activity, quantitating the  $\beta$  subunit is preferred because tumors may produce free  $\beta$  chains as well as intact hCG molecules.

#### *Membrane EIA assays*

Membrane EIA assays or antigen capture assay have made pregnancy testing easier, more specific, and less time consuming. In this procedure, monoclonal antibodies are attached to a filter membrane or another solid support. Urine or serum is passed through the filter membrane. Then it is incubated for a specific length of time. The hCG molecule binds to the antibody or is “captured.” An enzyme-labeled second antibody is added and binds to the hCG. The membrane is then carefully washed to remove all unbound enzyme-labeled antibody. A third reagent or substrate, which will produce a color reaction in the presence of the enzyme, is added to the membrane. Some assays read by viewing colored codes (a dipstick turning blue) or symbols such as plus (+) or minus (-). Certain pregnancy tests designed for public use will read “YES” or “NO.” This technique is rapidly gaining wide-spread popularity, since it is not only very sensitive, but it doesn’t require special equipment.

### **211. Syphilis testing**

The genus *treponema* contains both pathogenic and nonpathogenic species. Pathogenic treponemes are classified primarily upon the geographical location and the clinical manifestations of the respective diseases they cause. *Treponema pallidum* subspecies *pallidum* causes venereal syphilis; *T pallidum* subspecies *pertenue* causes yaws; *T pallidum* subspecies *endemicum* causes endemic syphilis; and *T carateum* causes pinta. Venereal syphilis is transmitted by sexual contact; the other diseases are transmitted through contact. Although there are two types of syphilis, venereal and endemic, this lesson will focus on venereal syphilis caused by *T pallidum* subspecies *pallidum*.

#### **General information on venereal syphilis**

*Treponema pallidum* ssp. *pallidum* (meaning pale thread) is the etiologic agent of venereal syphilis. The primary mode of transmission is by vaginal, anal and oral sexual contact. Although rare, kissing, sharing of needles and injections equipment, blood transfusion and accidental inoculation have been reported as routes of transmission. In tropical countries that still practice direct donor-to-person transfusions, syphilis can be transmitted through blood. However, in the United States, blood stored at 4°C for more than 72 hours is incapable of transmitting syphilis; because the spirochetes can’t survive at 4°C.

#### **Clinical significance**

Venereal syphilis has been on the rise in the last 10 years due to changes in sexual practices (e.g., homosexuality, increased number of sexual partners, etc.). It is usually in the top 5 of the most frequently reported communicable disease and is most common in the sexually active age group (15 to 30 years), nonwhites, and homosexual men.

#### **Stages of infection**

When spirochetes enter the body, they immediately begin to multiply and spread throughout the body via the circulatory system. The body initiates an inflammatory response that involves lymphocytes, plasma cells, and macrophages. It produces reagin, anticardiolipin antibodies, antitreponemal antibodies, and other immune complexes. If left untreated the infection usually progresses through very well defined stages. The stages are characterized clinically by a primary lesion, a secondary eruption involving the skin and mucous membranes, and finally, after a latent period, lesions of the skin, internal organs, bones, cardiovascular system, and central nervous system.

#### *Primary syphilis*

The primary lesion appears around 3 weeks after infection. At this time, diagnosis is accomplished by darkfield examination of fluid expressed directly from the lesion known as a chancre. Presence of spirochetes is diagnostic for the infection. Although cases have been reported with detectable levels

of antibodies early in the primary stage, a nonreactive test at this time does not rule out the disease. Nontreponemal and treponemal serologic tests are reactive in about 60 to 85% of the patients depending on the procedure. Usually, in untreated syphilis, after the apparent spontaneous resolution of the primary chancre, there is a period when no visible signs of the disease are present.

#### *Secondary syphilis*

This stage usually starts within a few months after the disappearance of the primary lesion. Occasionally, the secondary stage of syphilis may start before the primary lesion has disappeared. Secondary syphilis is characterized by headache, fever, sore throat, lymphadenopathy, lesions in the anal and genital regions, and a localized or generalized rash. All lesions are highly infectious! The lesions may last only a few days or up to a year. Periodic recurrences of these signs and symptoms may occur for a couple of years or until solid immunity develops. Immunity may take up to 4 years and 25% of untreated patients may be susceptible to repeated episodes of spirochetemia and metastatic infection. Diagnosis may still be made by direct darkfield examination of lesions. In addition, 90 to 99% of patients in this stage will have reactive serum serologic tests. With treatment, the symptoms of secondary syphilis disappear rapidly. Without treatment, symptoms eventually disappear and the disease usually enters a remission or latent period. However, the disease may progress directly from the secondary stage to the tertiary stage.

#### *Latent period*

The latent period isn't considered a distinct stage of syphilis. No signs or symptoms of syphilis are present during this period. The latent period may last for years or for the life of the patient. Nontreponemal serologic procedures may be nonreactive but treponemal procedures are reactive. In this stage, syphilis is no longer communicable, except transplacentally to a fetus during relapse episodes. If a reactive CSF treponemal test is observed in this period it is indicative of asymptomatic neurosyphilis or possibly a progression to tertiary syphilis.

#### *Tertiary (late) syphilis*

If latent period syphilis is left untreated, only about 15 to 20% of the patients will develop symptomatic tertiary syphilis. The manifestations of tertiary syphilis vary and will generally appear between 3 to 10 years. There are reports of tertiary syphilis seen 40 years after the onset of primary syphilis. Tertiary syphilis may involve the cardiovascular system, central nervous system, eyes, and ears. The most serious complication is the development of an aortic aneurysm which may rupture and result in immediate death. Initially, central nervous system disease is asymptomatic but can manifest itself as a seizure, stroke, slight or incomplete paralysis, dementia, delusional states, or personality changes. Syphilis infections in the eyes can cause interstitial keratitis and in the ears, may cause nerve deafness. In addition, although less frequent, lesions may be produced in the skin, bone, and internal organs. These noninfectious lesions are known as granulomas or gummas. Once again, the disease can be transmitted to a fetus. Generally, only treponemal procedures are reactive in this stage.

#### *Serologically related infections*

There are several spirochete diseases, which give false positive test results, used to detect the syphilis antibody. *Treponema pallidum* ssp. *pertenue* is the causative agent of Yaws (frambesia or pian). Yaws is a nonvenereal, systemic infectious disease, most commonly occurring in children in tropical regions, such as Equatorial Africa, West Indies, and tropical areas of the Americas and the Far East. *Treponema pallidum* ssp. *endemicum* is the causative agent of nonvenereal endemic syphilis. This disease is less serious than venereal syphilis, and is found mostly in children in developing tropical and subtropical countries of the world. *Treponema carateum* is the causative agent of pinta. Pinta is a form of treponematoses, in which chronic dyschromic dermatosis occurs. It is prevalent in parts of tropical America and characterized by the presence of spots on the skin, which may be white, coffee colored, blue, red, or violet. The disease is transmitted by direct person-to-person contact.

### Laboratory identification

It is hard to believe that 40 years ago the best way to confirm a diagnosis of syphilis was the *Treponema pallidum* immobilization (TPI) test. To perform the TPI, one had to infect the testicles of a rabbit with syphilis, necropsy the rabbit, harvest the spirochetes, and mix them with the serum of a patient to see if antibodies immobilized the spirochetes. Today, laboratory tests for syphilis fall under two categories: (1) nontreponemal and (2) treponemal procedures. Nontreponemal procedures are considered screening test. The rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) are the most commonly used nontreponemal procedures. Treponemal procedures are confirmatory test and include fluorescent treponemal antibody absorption test (FTA-ABS) and microhemagglutination *Treponema pallidum* (MHA-TP) which are the most popular. Please note, for patients with active lesions, darkfield microscopy is the test of choice for detecting syphilis.

### Nontreponemal procedures

Nontreponemal procedures detect a substance called reagin, where as, treponemal procedures detect actual antitreponemal antibodies. Reagin is found in the serum of patients infected with syphilis. It has the same properties as an antibody and in some text is referred to as a “nonspecific antibody.” The VDRL and the RPR use a colloidal suspension of extracts from animal tissue to detect reagin in serum or CSF. The animal extract suspension reacts with reagin to form visible masses known as flocculation (similar to precipitation and agglutination).

### RPR

The RPR is the standard screening technique used in Air Force clinical laboratories. You have probably performed it hundreds of times. Manufactured test kits consist of prepared reagents and plastic-coated testing cards. The reagent is a specially formulated cardiolipin solution, containing choline chloride for inactivation of interfering complement, and charcoal particles for visible agglutination. This test works well on serum, but plasma from a variety of anticoagulants is also acceptable. In the test, 0.05 ml of unheated patient serum is placed and spread within an 18-mm circle on the plastic-coated testing card using the droppers provided. Using a 20 gauge needle, a drop (1/60 ml) of the carbon containing antigen is added to each specimen and control circle. The card is placed on a slide rotator circumscribing a 3/4 inch circle for 8 minutes at 100 rpm. This rotation is best performed using a humidifying cover with a moist blotter to cover cards and prevent drying. The RPR reagents should be used at room temperature. It is possible that cold reagents result in hyposensitivity, and overly warmed reagents may result in hypersensitivity. Scratched cards may result in the gathering of the charcoal particles and appear to be flocculation.

Macroscopic flocculation of the carbon particles indicates the presence of reaginic antibodies in the serum, and the test is reported as reactive (positive). Absence of flocculation is reported as nonreactive (negative). Before storing the calibrated dropper, the needle should be blotted to remove charcoal, but not wiped, since this may remove the silicone coating. Because this procedure detects nonspecific type antibodies, biological false positives are common in the following conditions; systemic lupus erythematosus, rheumatic fever, viral or pneumococcal pneumonia, vaccinia, DPT immunizations, infectious mononucleosis, infectious hepatitis, leprosy, malaria, rheumatoid arthritis, pregnancy, and aging individuals. False negatives can result from poor technique, improper rotation, or bad reagents.

### Serum VDRL

The antigen used in this procedure is composed of cardiolipins extracted from beef hearts and lecithin. Cholesterol is added to the alcoholic mixture for increasing the antigen's effective reacting surface. The buffered diluent must have a pH range of between 5.9 and 6.1. The serum is inactivated by incubation at 56°C for 30 minutes. This inactivation is adequate for a 4-hour period. Control sera and specimens, once initially inactivated, require only a 10 minute inactivation for subsequent retesting. Cool all sera to room temperature before testing. A drop (from a calibrated 18-gauge needle) of antigen is added to 0.5 ml of heat inactivated serum on a slide with 14 mm ceramic rings.

The slide is then rotated for 4 minutes at 180 rpm. After rotation, the mixture is examined microscopically on low power (10 X magnification) for flocculation of the antigen particles. Flocculation indicates the presence of reaginic antibodies in the serum. Results are reported as reactive, weakly reactive, or nonreactive. Before any results can be reported, the control sera must produce valid results as outlined by the manufacturer. A quantitative VDRL is performed on all specimens yielding weakly reactive or reactive results in the qualitative test. This procedure is done by repeating the same procedure on dilute patient samples.

The reagents and apparatus employed in the qualitative test are likewise used in this procedure. Each dilution is treated as an individual serum. The highest dilution of serum producing a reactive (not weakly reactive) result is reported as the titer. Medical practice considers a reactive result, in the presence of clinical symptoms, as confirmatory evidence of syphilitic infection. However, in the absence of clinical findings, test reactivity can represent any of the following: latent syphilis, a biological false positive reaction, either temporary or chronic, or a technical or clinical error. A repeat test can be ordered and the titer on the second specimen can be compared to the first titer. A drop to nonreactive suggests a prior technical problem or a temporary biological false positive condition in the patient, whereas a rise in titer suggests the likelihood of syphilis. A stable titer remains inconclusive, requiring further medical follow-up as well as serological testing for treponemal antibodies. On the average, reagin does not become demonstrable until 6 to 8 weeks after infection. Once reagin begins to form, it usually increases rapidly until it reaches a peak during the secondary stage. After the secondary stage has ended, as a rule, the amount of reagin in the blood decreases until it reaches a stable level during the late course of the disease.

#### *Sources of error*

Listed below are a number of possible sources of error common in nontreponemal testing.

1. All reagents must be warmed to room temperature for testing. Cold reagents may decrease test sensitivity. If they are too warm, the test may be hypersensitive.
2. Biological false positives are possible.
3. Prozone reactions may occur; therefore, all weakly reactive, as well as “rough” reactions, should be titrated to detect potentially high titered sera.
4. Mix the antigen suspensions frequently to ensure an even suspension of antigen particles.
5. The pH of the buffered saline is critical; alterations may affect test sensitivity.
6. The VDRL antigen suspension should only be used on the day it is prepared.

#### *CSF VDRL*

For the CSF VDRL, the antigen is mixed with an equal volume of 10% saline to increase the sensitivity. A drop (0.01 ml) of this antigen mixture is added to 0.05 ml of unheated, clear spinal fluid in a 16 mm diameter by 1.75 mm deep concave well on a glass agglutination slide. The slide is rotated for 8 minutes at 180 rpm. After rotation, the mixture is examined microscopically at 10 X magnification for flocculation. Flocculation indicates the presence of reaginic antibodies. Before results can be reported, the control sera must produce the expected reactions. If the controls indicate valid results, report the patient’s spinal fluid as either reactive or nonreactive, as appropriate. **NOTE:** The “weakly reactive” report is not used for this test. Any reactivity is significant. The following sources are common for the CSF VDRL errors:

1. Be sure the VDRL antigen solution is mixed with equal parts of 10% saline.
2. The antigen drop of 0.01 must be accurate.
3. The reactive control sera should perform in predetermined ranges.
4. Spinal fluids containing gross blood or microbial contamination are unsatisfactory.
5. Maintain all reagents at room temperature for testing.



6. False positives rarely occur in tests for syphilis performed on CSF. A reactive test usually indicates present or past neurosyphilis, especially if the cell count and protein content of the CSF are elevated.
7. Understand that the spinal fluid is reactive in about one-fourth of the cases in the secondary stage of syphilis, thus indicating that central nervous system involvement has begun.

### ***Treponemal procedures***

Stating a patient has syphilis due to a positive screening test is not a good idea because of the many other biologic false positive conditions. Since the previously listed tests measure only nonspecific antibodies, we must confirm a possible syphilis infection with specific antibody procedures.

#### *Fluorescent treponemal antibody absorption test*

Earlier in this volume you reviewed the principles of fluorescent antibody testing, now, we will apply those principles. In the fluorescent treponemal antibody absorption test (FTA-ABS), heat inactivated patient's serum is first adsorbed with an extract of Reiter treponemes called *sorbent*. The sorbent reagent absorbs antibodies common to both saprophytic and pathogenic treponemes. The treated serum is added to the Nichols strain of *Treponema pallidum* that has been dried onto a glass slide and fixed with acetone. After incubation at 37°C, excess serum is rinsed from the slide with phosphate buffered saline (PBS). Next, a fluorescein labeled anti-human globulin (conjugate) is added to the *T. pallidum* smear, and the slides are incubated again at 37°C. At the end of this incubation period, excess conjugate is rinsed from the slide with PBS. The slides are coverslipped with a glycerine mounting medium and examined by fluorescent microscopy, using the appropriate exciter and barrier filters. The presence of Treponemal antibodies is indicated by a greenish fluorescence of the treponemes. The observations are compared with the fluorescence of known controls. False positive reactions have been reported in cases of systemic lupus erythematosus. Many of these demonstrate a peculiar "beaded" fluorescence of the treponemes. Other treponemal diseases (pinta and yaws) should also be expected to give reactive results. FTA-ABS antibodies usually appear in the serum during the primary stage of syphilis. Even after treatment, the test usually remains reactive for life, while most other serologic tests for syphilis become nonreactive, especially if treatment occurs before the tertiary stage of the disease. Test results are reported as reactive (positive) or nonreactive (negative).

#### *Microhemagglutination Treponema pallidum*

Microhemagglutination *Treponema pallidum* (MHA-TP) is the second most commonly run confirmatory test for syphilis; i.e., second only to the FTA-ABS. This test is finding its way into many labs due to its high degree of specificity (99%), ease of testing, and adaptability to microtiter plates, which allows for much larger testing loads. The test is a standard hemagglutination technique that uses sheep erythrocytes as a carrier for *T. pallidum* antigen. Similar to the FTA-ABS, the patient's serum is first absorbed with a sorbent made from nonpathogenic Reiter treponemes. Once absorbed, the serum is then allowed to react with sensitized sheep erythrocytes in a microtiter plate. Positive results exhibit a smooth mat (lattice formation) of erythrocytes. Negative test results fail to agglutinate, hence the erythrocytes fall out of solution and form a button in the bottom of the well.

#### *Other procedures*

Other nontreponemal screening procedures include unheated serum regain test (USR) and the reagin screen test (RST). Other hemagglutination treponemal confirmatory procedures include the hemagglutination treponemal test for syphilis (MATTS) and the *T. pallidum* hemagglutination assay (TPHA). Also, ELISA procedures have been developed for diagnosing syphilis. This procedure uses Nichols strain *T. pallidum* coated metal beads and a horseradish peroxidase-conjugated anti-human IgG as the second antibody. An enzyme substrate is added and results are read visually or spectrophotometrically for green color development.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 210. Routine tests performed

1. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
____ (1) Associated with a variety of infections (mycoplasma pneumonia and infectious mononucleosis).	a. Cold agglutinins.
____ (2) Titers of 1:16 or less are considered insignificant.	b. Primary cold agglutinin disease.
____ (3) B cell proliferation occurs with the production of plasma cells that produce heterophil antibodies.	c. Secondary cold agglutinin disease.
____ (4) Involved in skin infections (impetigo and cellulitis) and upper respiratory infections (e.g., pharyngitis).	d. Paroxysmal cold hemoglobinuria.
____ (5) Chronic, recurrent systemic inflammatory disease primarily involving the joints.	e. Infectious mononucleosis.
____ (6) Equipment used to draw blood must be pre-warmed to 37°C.	f. Streptococcal antibodies.
____ (7) The most common illness caused by the Epstein-Barr virus.	g. Rheumatoid arthritis.
____ (8) Antibodies may be produced to all these factors: antistreptolysin O, antistreptokinase, antihyaluronidase, and antideoxyribonuclease-B.	h. Systemic lupus erythematosus.
____ (9) Usually seen in the elderly and is managed by avoiding cold environments.	i. Pregnancy testing.
____ (10) Chronic systemic inflammatory autoimmune disease that affects multiple organs.	
____ (11) Monoclonal antibodies produced against the $\beta$ subunit must be obtained.	
____ (12) Antigenic stimulus activates the synovial B lymphocytes to produce an IgG that is recognized as foreign.	
____ (13) Heterophil antibodies that react with sheep, beef, and horse erythrocytes.	
____ (14) Marked by periods of painful acrocyanosis.	
____ (15) Complications include hemolytic anemia, aplastic anemia, encephalitis, Guillain-Barré syndrome, myocarditis, nephritis, and hepatic failure.	
____ (16) This is an IgG autoantibody that binds to P <sub>1</sub> and P <sub>2</sub> antigens, on human erythrocytes, at temperatures below 20°C.	
____ (17) Specimen must be kept warm, immediately transported to the laboratory, and placed into a 37°C waterbath or heat block.	
____ (18) Complications include rheumatic fever and acute glomerulonephritis.	
____ (19) Produces tissue damage within the joint (articular) and outside the joint (extra-articular).	
____ (20) Human chorionic gonadotrophin (hCG) is the hormone secreted.	
____ (21) ANAs are useful disease markers but have limited value in monitoring.	
____ (22) The disease follows a course of alternating exacerbations and remissions.	

**211. Syphilis testing**

1. What are the two types of syphilis?
2. How is venereal syphilis transmitted?
3. Why is venereal syphilis on the rise?
4. How are spirochetes spread throughout the body and what does the inflammatory response involve?
5. What are the stages of syphilis?
6. What is the characteristic of primary syphilis and how is it diagnosed?
7. What are the characteristics of secondary syphilis and how is it diagnosed?
8. What is the characteristic of the latent period and how is it diagnosed?
9. What are the characteristics of tertiary (late) syphilis and how is it diagnosed?
10. What is the most serious complication from tertiary syphilis?
11. What are the two categories of syphilis tests?
12. What are the two most common screening tests?
13. What are the two most common confirmatory tests?

14. Nontreponemal procedures detect what substance?
15. What does the RPR reagent contain?
16. In what conditions are RPR biological false positives common?
17. In the FTA-ABS, what does the sorbent contain and what is its purpose?
18. What treponemal strain is fixed to the glass slide?

## 3-2. Viral testing

Viruses are, of course, microorganisms. Because they are very difficult to cultivate, identification is primarily accomplished through immunological techniques. Most viruses produce measurable immune responses (i.e., antibodies). In this section, we will discuss the most commonly “tested for” viruses (with the exception of EBV because it was discussed earlier in this unit). Viruses are separated into families based on the type and form of their nucleic acid genome, size, shape, substructure, and mode of replication of the virus particle. The three key properties upon which the 73 families and groups are classified are the kind of nucleic acid (DNA or RNA), type of strandedness (single- or double-stranded), and the presence or absence of a lipoprotein envelope.

### 212. Hepatitis testing

Of the selected notifiable diseases in the *Morbidity and Mortality Weekly Report*, printed and distributed by the Massachusetts Medical Society, hepatitis (all viruses) usually only ranks behind Chlamydia, Gonorrhea, and AIDS in frequency. Hepatitis transmission, or lack of, should be a major concern for all health care workers because of its morbidity and mortality. At this time there are at least 5 hepatitis viruses: A, B, C, D, and E. We will study each of these viruses after a brief review of some general characteristics common to most viruses.

#### General characteristics of viruses

Viruses are a unique group of organisms, originally distinguished by their ability to pass through bacterial filters. But, size is only one distinguishing characteristic. Viruses consist of an inner core of nucleic acid, either DNA or RNA (never both), and an outer protein coat. Certain viruses are surrounded by an envelope containing cellular lipids and viral proteins (glycoprotein spikes). The entire virus is called the virion or viral particle.

#### Viral replication

Viruses lack enzymes and are unable to replicate outside a host cell. For this reason, they are referred to as obligate intracellular parasites. The steps or phases in virus replication is also known as the infectious cycle. This cycle can be divided into the following 9 very basic steps. Refer to figure 3-1 for an illustration.

1. Virus recognition by the host cells.

2. Viral attachment to the appropriate cell line.
3. Actual penetration into the cell.
4. Uncoating of the virus.
5. Transcription of viral DNA or RNA.
6. Protein synthesis of viral components.
7. Replication of viral DNA or RNA.
8. Assembly of components into new complete viral particles.
9. Release of virus from host cell.

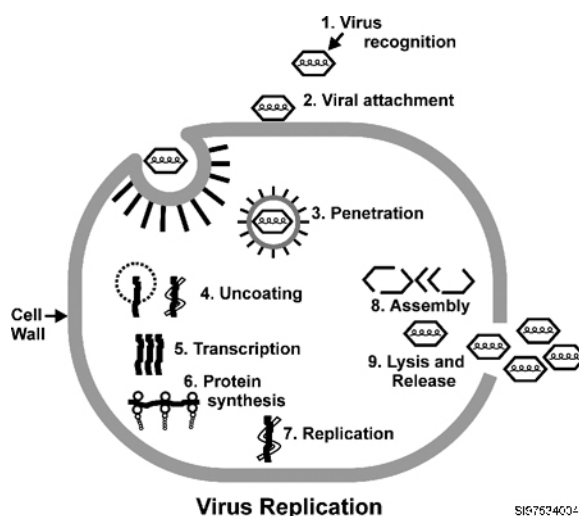


Figure 3-1. An illustration of viral replication.

### ***Process of phagocytosis***

A virus attaches to a host cell and is either engulfed into the cell through the process of phagocytosis as shown in figure 3-1, the viral envelope attaches to the cell membrane, or it injects or fuses its nucleic acid core into the cell. The latter two are shown in figure 3-2. Once inside the cell and free of its protein coat (uncoating), the nucleic acid (DNA or RNA) directs the host cell to manufacture the necessary enzymes and proteins required for viral replication. Protein components and newly manufactured nucleic acids are assembled into complete infectious virus particles by the host cell; according to the genetic code of the invading virus. The completed virus particles are then released from the cell through cell lysis, as shown in figure 3-1, by budding through the cell, or through envelopment. The latter two are seen in figure 3-2. Remember, a host cell can respond to a viral infection in three ways: (1) abortive infection or failed infection, (2) lytic infection or cell death, or (3) persistent infection or virus production without cell death. Hopefully, this brief review will come in handy in our study of viral testing. Now on to our hepatitis lesson.

### **Terms associated with hepatitis**

You should be familiar with the following terms and their association with the different hepatitis viruses.

### ***Hepatitis***

Hepatitis is the term used for inflammation of the liver. Hepatitis can be a result of alcoholism, amebic abscesses, autoimmune disorders, hepatotoxic drugs or chemicals, and viruses. Viral hepatitis is the most common liver disease reported worldwide. The viral agents responsible for hepatitis can be divided into two major categories: primary and secondary. The primary category contains hepatitis

A, B, C, D, and E viruses. These viruses are considered primary because they primarily attack the liver and have little direct effect on other body systems. The primary hepatitis category accounts for approximately 95% of all viral hepatitis cases. The secondary category consists of EBV, CMV, herpesvirus, and others. They are in the secondary category because liver inflammation is a consequence of systemic infection from these viruses.

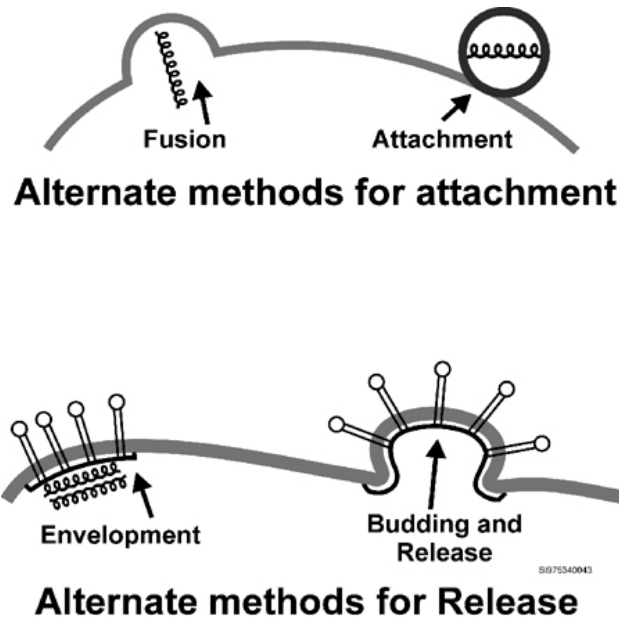


Figure 3-2. Alternate methods for attachment and release of viral particles.

### ***Acute hepatitis***

Acute hepatitis is typically associated with jaundice and a severe infection.

### ***Subclinical hepatitis***

Subclinical hepatitis is used to describe individuals with detectable antibodies in serum, but, there is no historical data for hepatitis or jaundice.

### ***Chronic hepatitis***

Chronic hepatitis lasts for 6 months or more is accompanied by hepatic inflammation and cirrhosis, and occasionally with hepatocellular carcinoma. Chronic hepatitis is known to occur with hepatitis B, C, and D, but not with A or E. However, only B and C are associated with hepatocellular carcinoma.

### ***Fulminant hepatitis***

Fulminant hepatitis is a rare form of hepatitis which results in hepatic failure with a high mortality rate and is associated with hepatitis D, B, and A.

### ***Icteric and anicteric***

Icteric means “pertains to, or affected with jaundice.” Jaundice is a syndrome characterized by hyperbilirubinemia and deposition of bile pigment in the skin, mucous membranes, and sclera resulting in a yellow appearance of the patient. Anicteric is a term for viral hepatitis “without jaundice.”

### **General information**

Viral hepatitis was first recognized as a disease in Europe and the United States during the late 18<sup>th</sup> and 19<sup>th</sup> centuries. During World War II, studies firmly established two types of hepatitis now labeled

hepatitis A and hepatitis B. Blumberg and associates discovered the “Australia antigen” which had been formerly called the “hepatitis-associated antigen (HAA),” now known as the hepatitis B surface antigen (HBsAg). The discovery of HBsAg was a major step in the differentiation of hepatitis B (HBV) from the other forms of viral hepatitis.

### **Clinical significance**

The symptoms of the various hepatitis viruses range from asymptomatic to death.

#### ***Hepatitis A virus (HAV)***

Approximately 40% of all acute hepatitis infections are due to HAV. HAV is transmitted from person-to-person through the fecal-oral route or by exposure to contaminated food or water. HAV incubation is from 15 to 40 days. The virus may be found in the stool of infected patients. Outbreaks are generally a result of contaminated food or water supply. The outbreaks can usually be traced back to an infected food handler, or, if eaten, shellfish from contaminated water. Major sources of spreading are day-care centers, crowded living conditions, and poor personal hygiene. Most children with HAV are asymptomatic. However, adults have nausea, vomiting, dark urine, abdominal pain, and fatigue. Abdominal tenderness, hepatomegaly, and jaundice may also be seen. It is believed that liver damage is due to immune responses to the virus and not the virus itself. Although HAV can lead to fulminant hepatitis, chronic hepatitis and a chronic carrier state doesn't occur. HAV is a very hearty organism that can survive exposure to an acid pH of 3, ether, chloroform, detergents, saltwater, drying, and varied temperatures. At the time of this writing, there is a multi-state outbreak of school age children with HAV. These children acquired the infection from contaminated frozen strawberries served at lunch in the cafeteria. Also, the HAV vaccine just became available and mandatory for certain military personnel.

#### ***Hepatitis B virus (HBV)***

HBV infections can be asymptomatic to symptomatic, and acute or chronic. Half of the infections exhibit no symptoms or mild nonspecific viral illness, and are anicteric. HBV is transmitted by parenteral (injection), sexual contact, or mother-to-infant modes. Incubation is between 15 to 150 days. It's spread through sharing needles, acupuncture, ear and body piercing, tattooing, and body secretions. HBV has been found in saliva, amniotic fluid, breast milk, semen, and vaginal fluids, but the major source of infection is blood and blood products. Individuals at risk for HBV are those with multiple sex partners, drug users, homosexual men, infants born to HBV positive mothers, dialysis patients, recipients of blood or blood products, and some health care workers. Patients at risk for chronic infections include infants, immunosuppressed patients, patients with lymphoid cancer, and children with Downs syndrome. Children ordinarily have milder symptoms than adults. If symptoms are present, 95% of the patients will have malaise, 90% anorexia, 80% nausea, 15% fever, rash, or arthritis, and 10% have itching.

Acute HBV infections may produce an enlarged liver, abdominal tenderness, and splenomegaly. It is also believed that liver damage is due to an immune response in which lymphocytes derived from the liver or circulation are cytotoxic to the hepatocytes of the liver. The mortality for hepatitis B is much greater than that for hepatitis A. Approximately 5 to 15% of patients with HBV will develop a chronic infection and 10% of these patients may develop cirrhosis or liver failure. Individuals with chronic HBV are a major source for spreading the virus. They are also at risk for developing fulminant hepatitis if co-infected with the HDV. The World Health Organization estimates that 80% of cases of primary hepatocellular carcinoma are a result of chronic hepatitis B infections. Primary hepatocellular carcinoma is frequently fatal and is one of the three most common causes of cancer mortality in the world.

#### ***Hepatitis C virus***

Hepatitis C virus (HCV) (formerly non-A, non-B) accounts for 90% of all non-A, non-B hepatitis (NANBH) infections and is the major cause of post-transfusion hepatitis. It can bring about an acute

or chronic infection. HCV was not identified until 1989 when Choo et al isolated the cDNA clone from the blood of a patient with NANBH. Worldwide distribution is relatively uniform ranging from 0.3 to 1.5% of blood donors. Therefore, the major known mode of transmission is blood. Incubation varies from 14 to 180 days. Because it is transmitted through parenteral and sexual contact, the spreading of HCV, and the individuals at risk for HCV, are the same as for HBV. However, it doesn't seem to transfer from mother-to-infant during pregnancy. The symptoms of HCV are very similar to those of hepatitis B. Chronic infection is even more prevalent (about 50% of infected patients) for HCV than for HBV. It often leads to acute disease, cirrhosis, and, occasionally, to fulminant hepatitis and primary hepatocellular carcinoma. There have been reports of sporadic, community-acquired hepatitis C but the mode of transmission is unknown at this time.

### ***Hepatitis delta virus***

The delta virus infects approximately 15 million people worldwide and is responsible for 40% of fulminant hepatitis cases. Hepatitis delta virus (HDV) is sometimes called a "viral parasite" or "defective virus" because it can only replicate in HBV-infected cells. Transmission of HDV is through parenteral and sexual contacts. The incubation period ranges from 15 to 65 days and is spread by blood, semen, and vaginal secretions. Because HDV and HBV are transmitted by the same routes, an individual can be *co-infected* with HBV and HDV at the same time. Both infections begin at the same time and can be acute or fulminant. In addition, *superinfections* occur when a person with chronic hepatitis B is infected with HDV at a later date. The delta agent increases the severity of hepatitis B infections and its symptoms. An infection with HDV is suspected in patients with fulminant hepatitis and in chronic HBV carriers who experience a sudden deterioration in their clinical condition. HDV superinfections can cause severe acute or chronic hepatitis infections. Also, HDV has been responsible for some epidemics with high mortality rates.

### ***Hepatitis E virus***

Hepatitis E virus (HEV) is a major cause of acute hepatitis resembling hepatitis A. It is spread via the fecal-oral route and produces both sporadic and epidemic hepatitis in developing countries. At the time of this writing, the only cases found in the United States have been found in travelers from endemic areas (i.e., southern Italy, Amazon basin, parts of Africa, and the Middle East). Infections reported in North America and Western Europe usually occur in illicit drug users. Clinical symptoms are very similar to those presented by HAV. HEV doesn't lead to chronic hepatitis and is not associated with hepatocellular carcinoma. Nevertheless, an interesting phenomenon seen in HEV infections is an unexplained high fatality rate among pregnant women.

### ***Viral markers***

Hepatitis is identified and diagnosed through the use of serologic markers. These antigens and antibodies can be assayed and used extensively to follow the course of disease. Each virus produces characteristic antigens and antibodies related to the physical properties of the virus itself.

### ***Hepatitis A virus***

The hepatitis A virus (HAV) is a small capsid RNA virus of the picornavirus family. This particular virus doesn't have core or envelope structures. It is approximately 27 to 29 nm in diameter. As shown in figure 3-3, HAV has a single-stranded RNA genome with about 7478 nucleotides and a genomic viral protein (VPg).

### ***HAV antigens***

Although possible, the HAV antigen (infectious virus) is difficult to detect in stool. Efficient tissue culture systems for growing the virus are not available and the virus may be shed before symptoms are observed. Acute hepatitis A infections are usually diagnosed through serologic techniques that detect HAV antibodies.



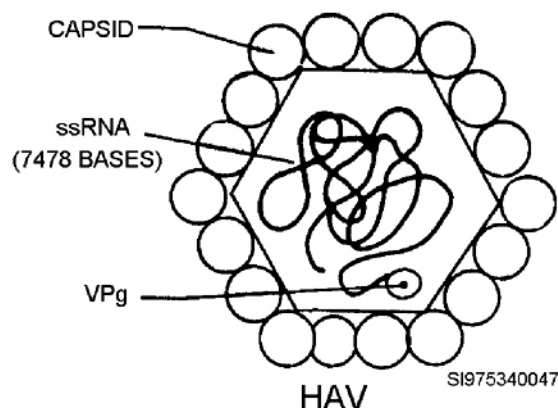


Figure 3-3. Illustration of HAV.

#### *HAV antibodies*

There are two serologic markers that can be assayed to aid in the diagnosis of HAV: anti-HAV IgM and anti-HAV IgG. The presence of anti-HAV IgM in serum represents a recent hepatitis A infection. IgG antibodies are long-lived and may be present for life. Anti-IgG represents an immunity to infection.

#### *Hepatitis B virus*

Hepatitis B virus (HBV) is a very complex, small enveloped DNA virus and is a member of the new hepadnaviruses. It is a double-shelled particle referred to as the *Dane particle*. It measures about 42 nm in diameter. As shown in figure 3-4, the virus has an outer coat or surface, known as the hepatitis B surface antigen (HBsAg). Next to the outer surface, is an inner core compartment called the hepatitis B core antigen (HBcAg). Hepatitis Be antigen (HBeAg) protein is a minor component of the virion and shares part of its protein sequence with HBcAg. Just inside the inner core are a DNA polymerase, a protein kinase, and the viral genome. The genome is small, round, *partly* double-stranded DNA (one of the strands is incomplete) with 3200 nucleotides. The DNA polymerase completes the single-stranded area of the DNA, along with the protein kinase, is needed for viral replication.

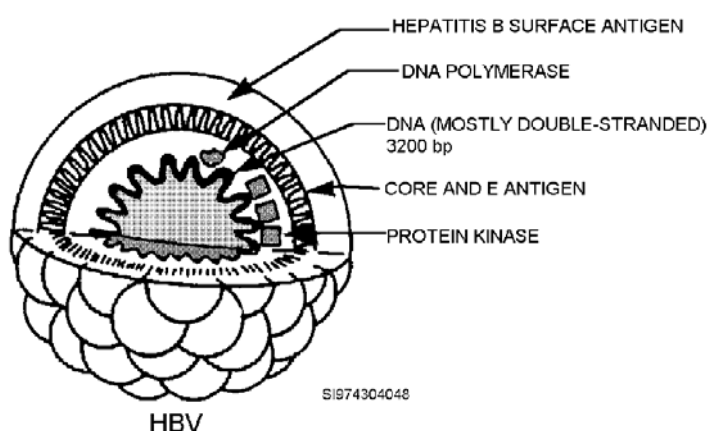


Figure 3-4. Illustration of HBV.

#### *HBV antigens: HBsAg and HBeAg*

Hepatitis B surface antigen (HBsAg) is the first serologic marker to appear after infection with HBV and can be detected in the serum during the incubation phase of the disease. During the acute phase of the disease, HBsAg titer parallels liver enzyme values as they increase. Although some patients completely clear HBsAg before the onset of symptoms, this antigen usually disappears about the time that liver studies return to normal. Presence of HBeAg is indicative of active replication of the hepatitis virus and identifies the patient as highly

contagious. This antigen first appears about the same time as HBsAg, but it is extremely unstable and persists for a only a short period of time. HBcAg is usually only detectable in liver cells, and only in rare cases is it found in peripheral blood.

### *HBV antibodies*

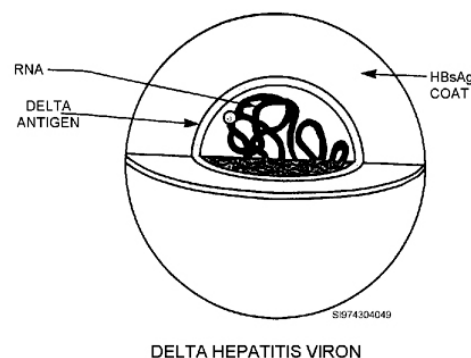
There are four antibodies, which may be tested as serological markers for HBV infections. The disappearance of the antigen (HBeAg) is followed immediately by the appearance of its antibody—anti-HBe. This antibody response (seroconversion) occurs normally at the peak of clinical symptoms and is indicative of resolution of the disease. The detection of anti-HBe, along with the rapid disappearance of HBeAg, is usually synonymous with probable recovery and lessening of the patient's infectious state. However, the patient is still considered infectious as long as HBsAg is detectable. Anti-HBc is produced in response to the HBcAg. Anti-HBc appears very quickly after the original onset of symptoms. This usually occurs about 1 to 4 weeks after the appearance of HBsAg in the serum. When anti-HBc is first detected, this IgM subclass antibody will appear and last up to 6 months. Then, it disappears after the onset of symptoms. The characteristics of anti-HBe and anti-HBc IgM are used to diagnose recent acute cases of hepatitis B. Anti-HBc IgG antibody quickly replaces this IgM subclass antibody as it disappears from the circulation. Presence of anti-HBc is indicative of either an active HBV infection or a past infection. Anti-HBs does not appear during the acute phase of the disease, rather it manifests during the convalescent period. In most patients, anti-HBs will appear only after HBsAg has disappeared and total recovery is realized. Anti-HBs is, therefore, called the “*marker of recovery*” or immunity. This long-lived antibody is the major protective antibody against HBV infection. Hence, the success of the HBV vaccination.

### *Hepatitis C virus*

Hepatitis C virus (HCV) is an enveloped, single-stranded RNA virus with 9500 nucleotides. It is a member of the flavivirus group and measures about 45 nm in diameter. This group also contains dengue and yellow fever viruses. The only definitive test to detect HCV (antigen) is through reverse transcription and polymerase chain reaction (PCR) amplification of viral sequences. At this time, these procedures are available only as a research tool. Synthetic proteins derived from the cDNA sequence quickly led to the development of anti-HCV assays, even before HCV was completely isolated. Anti-HCV can be detected within 6 to 8 weeks of infection. A positive anti-HCV test suggests a chronic rather than an acute infection.

### *Hepatitis D virus*

As shown in figure 3-5, Hepatitis D virus (HDV) has a very small single-stranded RNA genome with 1700 nucleotides. The virion is approximately 35 to 37 nm in diameter. The genome is surrounded by the delta antigen that is encircled by an HBsAg-containing envelope.



**Figure 3-5. Illustration of HDV.**

### *Hepatitis E virus*

Hepatitis E virus (HEV) is about 27 to 34 nm in diameter with a single-stranded RNA genome. It is a calicivirus. HEV RNA has been detected in stool by PCR assays. Anti-HEV can be detected by ELISA procedures.

### **Laboratory identification**

Doctors and laboratory technicians alike have been confused by the myriad of techniques available for the diagnosis, treatment, and monitoring of viral hepatitis. Each methodology has its merits and associated proponents. In the Air Force, the most accepted technique is the use of panel testing. As in any panel, a standardized set of tests is prescribed and used for each diagnostic purpose. These panels will be dictated by your laboratory or reference laboratory; check your OIs for details. Along with liver enzymes, RIA, ELISA, and micro-membrane procedures are used for identifying and monitoring hepatitis infections.

### **213. Human Immunodeficiency Virus testing**

Acquired immune deficiency syndrome (AIDS) was first recognized in 1981, but it wasn't until 1983 – 1984 that the human immunodeficiency virus (HIV) was identified as the causative agent of AIDS. Since that time, our knowledge about the virus and immune system has increased tremendously. Nonetheless, the origin of the characteristics and the profound immune dysfunction caused by HIV still remains a mystery. In this lesson, we will briefly discuss some of what is known about HIV.

#### **General information**

In the late 1970s and early 1980s, it was noted that an unusual number of young homosexual men, heroin addicts, hemophiliacs, and Haitians were dying of normal benign opportunistic infections. The symptoms from these groups of individuals lead to, as well as, defined a new disease known as AIDS. Today AIDS is not limited to these groups. There are more than a million people worldwide who currently have AIDS and it is estimated that over 8 million people have been infected with HIV. At this time, all HIV infected persons whose disease progress to AIDS are expected to die of the complications and/or opportunistic infections common to the disease. The largest number AIDS cases are reported in the Americas and Africa.

#### ***Modes of transmission***

HIV is transmitted through sexual contact, infected blood and blood products, contaminated needles or syringes, artificial insemination with infected semen and from mother-to-infant during pregnancy, birth, or breast feeding. HIV has been detected in blood, semen, vaginal secretions, saliva, urine, feces, sweat, tears, and amniotic fluid. However, all but blood, semen, and vaginal secretions, are considered unlikely to transmit HIV because of their extremely low viral titers. In the United States, Europe, and Australia, homosexual activity accounts for the majority of sexually transmitted cases. High risk groups (in these countries) include homosexual and bisexual men, intravenous drug users, multi-partner sexually active persons, prostitutes, and children born to infected mothers.

#### ***Before 1985***

Before 1985 (the beginning of routine screening), blood and organ transplant recipients and hemophiliacs were also considered at high risk for HIV. Those in the low risk group are health care workers (nurses, doctors, dentists, laboratory staff, etc.). In Africa, AIDS is equally distributed among women and men. Heterosexual contact, specifically from infected female prostitutes to their clients, is believed to account for most of the HIV infections. In Africa, high risk factors include having large numbers of sexual partners, prostitution, having sex with prostitutes, history of other sexually transmitted diseases, genital ulcerations, multiple uses of needles and syringes in health care facilities, and using un-sterilized instruments in ritualistic customs (scarification, tattooing, and ear piercing).

#### **Clinical significance**

HIV exposure doesn't always lead to infection, but it only takes 1 exposure of the right inoculum size, mode of transmission, or host factors to produce an infection. About 50% of HIV infected individuals will develop AIDS within 10 years of exposure. HIV infections range from an asymptomatic infection to a profound immunosuppression known as "full-blown AIDS." The time from exposure to the onset of disease depends on the viral inoculum, virulence of strain, mode of transmission, and age of the patient. HIV infections have been classified according to serological results, clinical symptoms, presence of opportunistic infections or certain cancers, and CD4 lymphocyte depletion. Asymptomatic, acute HIV mononucleosis, AIDS-related complex (ARC), and AIDS may be used to describe the classifications of HIV infection.

#### ***Asymptomatic***

After exposure to HIV, an individual may not exhibit any signs of HIV infection but have a reactive or positive viral marker (HIV antigen or antibody). However, most infected individuals will progress to an acute illness that resembles infectious mononucleosis.

### Acute HIV mononucleosis

Acute HIV mononucleosis generally develops within 2 to 6 weeks after infection, with reported periods ranging from 5 days to 3 months. Acute HIV mononucleosis symptoms are fever, headache, sore throat, fatigue, body discomfort, rash, lymphadenopathy, hepatosplenomegaly, and possibly neurologic disease.

### AIDS-related complex

AIDS-related complex (ARC) is a somewhat inadequately defined condition. ARC encompasses patients who are chronically ill, but fall somewhere between acute HIV mononucleosis and the Center for Disease Control and Prevention's criteria for AIDS. Symptoms consist of a persistent fever, night sweats, weight loss, unexplained chronic diarrhea, eczema, psoriasis, and generalized lymphadenopathy. If herpes zoster, oral candidiasis, and oral hairy leukoplakia are seen, it's indicative of the infection progression to AIDS.

### AIDS

The Center for Disease Control and Prevention has developed diagnostic criteria for full-blown AIDS, which involves certain opportunistic infections and cancers, HIV-related encephalopathy, HIV-induced wasting away syndrome, and laboratory evidence of infection. The opportunistic infections are *Pneumocystis carinii* pneumonia, toxoplasmosis, *Mycobacterium avium* complex disease, disseminated *Mycobacterium tuberculosis* infection, disseminated cytomegalovirus infection, and persistent ulcerative herpes simplex virus infection. AIDS patients also have a higher incidence of *Salmonella* bacteremia, staphylococcal infections, and pneumococcal pneumonia. The most common cancers seen are Kaposi's sarcoma, neoplasms of the skin, and B cell lymphomas. Laboratory evidence is a CD4 lymphocyte count of less than 200 cells/ $\mu$ l or a CD4 T lymphocyte level of <14% of total lymphocyte count.

### Viral markers

HIV is a member of the retroviruses which are rough, spherical enveloped RNA viruses that possess the enzyme reverse transcriptase. Reverse transcriptase allows the virus to synthesize a DNA copy of its RNA genome. The HIV virion measures from 80 to 120 nm in diameter. It consists of an inner core, protein shell, and a lipid bilayer envelope as shown in figure 3-6. The inner core contains two identical RNA strands and reverse transcriptase. The genome consists of at least 3 major genes that encode for enzymatic and structural proteins of the virus. These genes are the *gag* (group-specific antigen), *pol* (polymerase), and *env* (envelope). The HIV genome carries within it the genetic information required to reproduce HIV viruses within the host cells. The viral envelope contains glycoprotein spikes and HLA antigens that are acquired by the budding of the virus from the cells plasma membrane.

### Molecular weight

The names of the proteins (p) and glycoproteins (gp) are derived from their molecular weight. For example; within the protein shell is a core protein that weighs approximately 24,000d and is known as p24. The glycoprotein spike has two parts the "stick" area that crosses the viral bilayer and the

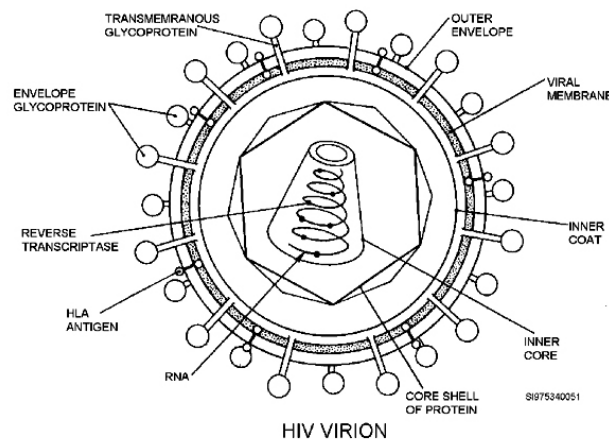


Figure 3-6. Illustration of HIV virion.

“knob-like” area attached to the stick. These are known as gp41 and gp120 respectively. Each of these proteins and glycoproteins are antigenic.

### ***Viral replication***

Refer to figures 3-1 and 3-2 for this text. Replication of HIV is by the binding of the viral glycoprotein spikes to cell surface receptor proteins. The virus enters the cell by fusion of the envelope with the cellular plasma membrane. HIV is uncoated and the exposed viral RNA is transcribed into DNA and inserted into the host’s cell genome during cellular division or activation. Once activated, transcription of the virus occurs. Next protein synthesis, replication, and assemble of a mature virion takes place. The virion escapes the host cell by budding from the cellular membrane. When HIV replication occurs, CD4-containing cells are killed, resulting in a severe depletion of cells, especially helper T cells.

### **Laboratory identification**

During the first or acute stage of the infection, the HIV p24 (core protein) antigen can be detected using enzyme immunoassays, but only for a short time. The first antibody to usually appear in the serum is anti-gp120, closely followed by anti-p24. They appear 2 to 8 weeks after the onset of infection. Keep in mind that during seroconversion, the various antibodies directed against the viral proteins do not develop simultaneously. Once anti-p24 appears the HIV p24 antigen rapidly declines. The ELISA is the most popular screening procedure. Purified whole virus is broken apart and the viral proteins are attached to plastic beads or the wells of microtiter plates. Patient serum and controls are added to the wells. HIV antibodies will bind to the viral proteins. An enzyme-linked anti-human antibody will bind to the complex. After the addition of an appropriate substrate, a color reaction will take place and is measured colorimetrically. A repeat ELISA is performed on all positive test serums. If the sample repeats positive a confirmatory test is performed. Confirmatory test include Western blot, immunofluorescence assays, or ELISA with recombinant antigens. The widely used Western blot detects specific antibodies directed against the HIV proteins and glycoproteins. As with the ELISA, the virus is broken apart and the proteins are electrophoresed on a polyacrylamide gel, transferred to a nitrocellulose membrane, and then reacted with test serum. An enzyme-linked anti-human globulin is added to the membrane, a substrate is then added, and a color develops on the membrane strips. The “bands” of color represent a different viral protein. The Western blot test measures p17, p24, p31, gp41, p51, p55, p66, gp120, and gp160. Recent Center for Disease Control and Prevention criteria indicates that an HIV infection is confirmed if there is a response to any two of the following; p24, gp41, or gp120/160. Because neonates will passively acquire anti-HIV antibodies, which may be present for up to 15 months, they pose a difficult serodiagnostic problem. Culture of the actual virus from blood or tissue, or PCR HIV genome amplification may be necessary to confirm HIV infection in asymptomatic infants.

## **214. Miscellaneous viruses**

This lesson is dedicated to the miscellaneous viruses that cause human infection. Let’s divided these viruses into two very broad categories: (1) viruses that infect various host cells, and (2) viruses that mainly infect the cells of the immune system. The viruses included in category 1 are hepatitis, rubella, measles, mumps, respiratory syncytial virus (RSV), influenza, parainfluenza, rabies, and poliovirus. Category 2 consists of the human immunodeficiency virus, Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV-1 and -2), varicella zoster virus (VZV), human T cell leukemia virus types I and II (HTLV-I and HTLV-II), and human herpes virus type 6 (HHV-6). We will not be able to discuss all of these viruses in-depth, but we will look at them briefly.

### **Rubella (German measles)**

In Latin rubella means “little red.” It was distinguished from the other measles by German physicians, hence the name *German measles*. Rubella is spread through respiratory secretions BY aerosols and contact modes. It generally acquired in childhood and is promoted in crowded conditions such as

daycare centers. The incubation period is between 17 and 20 days. First, the virus infects the upper respiratory tract and spreads to the local lymph nodes. This corresponds with a period of lymphadenopathy. The virus then enters the blood stream (viremia) and is dispersed throughout the body. Infection occurs in different tissues and the typical mild rash results. A lifelong immunity exists from just 1 infection. Interestingly, the secretory IgA in the respiratory tract plays an extremely important role in the protection against a secondary challenge with rubella.

### ***Clinical significance***

This infection usually causes a mild exanthematous childhood disease, but has serious consequences for the neonate. In children, it exhibits a 3-day rash and swollen glands. In adults, it is usually more severe leading to arthritis and arthralgia. Although rare it may lead to post-infections encephalitis and thrombocytopenia.

### ***Pregnant mothers***

Although rubella can reach epidemic proportions in children and young adults, it is the pregnant mother who must protect her unborn child from the gravest danger of this infection, especially in the first trimester. If a pregnant mother doesn't possess the antibody, the virus can replicate in the placenta and spread to the fetal blood supply. From the blood supply it is spread throughout the small fetal body and replicates in most of its tissues. The virus doesn't kill fetal tissue cells, but effects the normal growth, mitosis, and chromosomal structure. This leads to small birth size, improper development, and other physical defects. Maternal rubella infections are associated with several severe congenital defects. Clinical manifestations of congenital rubella infections are cataracts, mental retardation, deafness, heart defects, hepatomegaly, bone defects, and failure to thrive. Severely afflicted children have multiple defects. There is a high mortality for these babies in utero and in the first year of life.

### ***Laboratory identification***

Rubella is a member of the togaviruses and the incubation period varies from 10 to 21 days. It is an enveloped, single-stranded RNA virus. A serum titer for the antibody to the rubella virus is one of the most important tests we can perform on females of childbearing age. In the 1<sup>st</sup> to 4<sup>th</sup> week of infection, IgM is detectable. IgG antibodies begin within 7 to 21 days, increase rapidly, then decline, but are present and provide protective immunity indefinitely. Hence, the detection of IgG is only an indication of a recent infection when testing paired specimens. A four-fold increase in titer is indicative of a recent infection.

### ***Procedures***

The two types of tests commonly performed to evaluate serum rubella titer are hemagglutination inhibition (HAI) and rapid agglutination card tests. In the HAI procedure, the patient sample is diluted in a microtiter plate; rubella antigen is then added to all wells, followed by erythrocytes which are used as an indicator. Dilutions, which contain rubella antibodies, will show no agglutination. The last dilution that shows no agglutination is the serum titer. A titer of 1:8 or greater is considered sufficient rubella immunization. A titer of <1:8 is best followed by a repeat sample about two weeks later. The rapid agglutination card test is a latex screening procedure that detects both IgM and IgG. In order to confirm a recent infection EIA and IFA specific for IgM must be performed. **NOTE:** IgM can be detected for a few weeks after a rubella immunization.

### ***Immunity***

If the virus is acquired by the mother early in her pregnancy and she transmits it to the developing fetus, various birth defects or a spontaneous abortion can be expected. It then becomes the role of the clinical laboratory to determine the immune status of women during their prenatal health care. If a pregnant woman who is susceptible to rubella is exposed to, or contracts the disease, the laboratory can expect to run frequent rubella titers to determine potential risk to the fetus. Infants born of mothers with immunity have a passive immunity that will last for about six months. To acquire

permanent immunity, vaccinations with an inactivated live virus or suffering through the disease are the only viable methods. Vaccination imparts immunity by causing a mild infection. For this reason, females of childbearing age are strongly encouraged to practice birth control for two months following immunization. We also need to stress that immunized individuals may still transmit the virus to pregnant females.

### **Measles**

Measles virus is in the family paramyxovirus. It induces a potentially serious generalized infection characterized by a maculopapular rash known as *rubeola*. It is also responsible for a chronic slowly progressive neurologic disease known as subacute sclerosing panencephalitis. Measles is a highly contagious infection and incubation ranges from 9 to 11 days. It is transmitted from person-to-person by respiratory droplets through the airborne route. It replicates in the respiratory tract, spreads to the lymph nodes, and then to the blood. Once disseminated the virus causes infection of the conjunctiva, respiratory tract, urinary tract, small blood vessels and the central nervous system. The rash is caused by immune T cells targeted to measles-infected endothelial cells lining small blood vessels. Pneumonia, otitis media, and bacterial superinfections are complications from the measles virus. Most patients recover and have lifelong immunity, but some develop post-infectious encephalitis.

### **Mumps**

Mumps is also a paramyxovirus. It infects epithelial cells of respiratory tract and causes painful swelling of the salivary glands. It is also known as *epidemic parotitis*. Viremia spreads the virus to the testes, ovaries, pancreas, thyroid, other organs, and CNS (meningoencephalitis). It is spread from person-to-person by respiratory droplets, direct contact, and through fomites. The MMR vaccine provides immunity against the measles, mumps, and rubella viruses. Because of unvaccinated individuals, immunocompromised individuals, and those in developing countries this virus is still active.

### **Respiratory syncytial virus (RSV)**

Respiratory syncytial virus (RSV) is the most frequent cause of fatal acute respiratory infection in infants and young children with cardiorespiratory conditions. It produces an upper respiratory infection in adults and severe pneumonia and bronchiolitis in children. RSV infects virtually everyone by 4 years of age, reinfections occurs throughout life and even among the elderly. RSV is very contagious and is transmitted on hands, fomites, and occasionally respiratory routes. If this virus is introduced into a nursery or intensive care nursery, it can be devastating. A vaccine was under development, but it brought about a more serious disease, so at this time there is no vaccine.

### **Influenza A and B**

Influenza A and B cause a local upper respiratory tract infection. Influenza A infects both humans and animals but B has no animal reservoir. Influenza outbreaks occur annually and remain present in a community between 4 and 6 weeks. It is spread by fomites and small airborne droplets released during talking, breathing, and coughing. This hearty virus can survive for 24 hours on a countertop. The most susceptible groups of people are school age children, immunosuppressed individuals, pregnant women, elderly persons, those with heart and lung ailments, and smokers. These groups are also at a higher risk for a more serious infection, pneumonia, Reye's syndrome (acute encephalitis), and CNS involvement. The mortality rate is highest in the elderly and in individuals with underlying chronic cardiorespiratory disease. Many flu-like symptoms (fever, nonproductive cough, fatigue, body aches, and headaches) are seen with these viruses. Incubation is 11 to 14 days.

### **Parainfluenza**

Parainfluenza is a respiratory virus that usually causes mild cold-like symptoms, but can bring about a serious respiratory disease. Inhalation of large droplets from aerosols is the mode of transmission. It produces bronchiolitis, pneumonia, and croup in small children.

**Rabies**

Rabies is usually a fatal disease unless treated by vaccination. It is spread through infected dogs, cats, skunks, raccoons, foxes, bats, and other wild and domestic animals. Following the bite, the virus replicates in the muscles and nerves. Traveling through the nerves it finally reaches the spinal cord and CNS. Symptoms include hyperexcitability, hydrophobia (fear of water), gastrointestinal tract disease, fatigue, and anorexia. Identification is made by detecting the antigen in the CNS or skin by immunofluorescence.

**Poliovirus**

Polio is transmitted through the fecal-oral route and is spread by poor hygiene, dirty diapers, and ingestion of contaminated food and water. The poliovirus causes a poliomyelitis and an acute encephalomyelitis. This disease has been virtually eliminated in vaccinated populations. However, the live vaccine (used in the United States) may actually, although rarely, revert to virulence, producing a paralytic disease in the vaccinee or their contacts. Young children usually exhibit an asymptomatic or mild disease; however, it can progress to a paralytic disease.

**Cytomegalovirus**

Cytomegalovirus (CMV) is a member of the herpes virus family, which includes EBV, HSV-1 and -2, VZV, and HHV-6. CMV is the most common viral cause of congenital defects and is a pathogen in immunocompromised patients. CMV establishes latent infection in mononuclear lymphocytes, stromal cells of the bone marrow, and other cells. It is spread by blood transfusions and organ transplants, as well as, through other body fluids. Interestingly, a CMV infection is immunosuppressive because it induces a decrease in helper cells (CD4) and an increase in suppressor cells (CD8). Congenital infections result in unilateral or bilateral hearing loss, dental defects, and mental retardation. About 20% of pregnant women in the United States harbor CMV in their cervix. If a premature infant acquires CMV from birth or blood transfusion, pneumonia and hepatitis are commonly seen infections. Cytomegalic inclusion disease (CID) is the term used for infants infected with CMV. Clinical features of CID are hepatosplenomegaly, microcephaly, chorioretinitis, thrombocytopenia, and jaundice. Approximately 50% of adults by age 35 are positive for CMV. It is spread by saliva, close personal contact, and sexual contact because it is present in the cervix and semen. Most infections are acquired in young adults and are generally asymptomatic. However, clinical symptoms resemble infectious mononucleosis caused by EBV, but with less severe pharyngitis and lymphadenopathy.

Fever, splenomegaly, a mild pneumonia, and hepatitis are often seen in those infected with CMV. It also gives rise to atypical lymphocytes, which can be seen, on peripheral blood smears, but in contrast to EBV, heterophil antibody tests are negative. CMV is an opportunistic infection in immunocompromised individuals, and in these patients it results in retinitis, interstitial pneumonia, encephalitis, colitis, and esophagitis. CMV, like the other herpes viruses, can induce a persistent latent recurrent infection.

**Herpes simplex virus (HSV-1 and HSV-2)**

Herpes simplex viruses produce cold sores and genital lesions. HSV-1 is usually associated with infections above the waist and HSV-2 below the waist. HSV-2 has a greater potential to cause viremia and associated flu-like symptoms. HSV-1 and -2 are transmitted through semen, saliva, and vaginal secretions. Both types can cause oral or genital lesions. HSV-1 is usually spread by kissing, sharing drinking glasses or toothbrushes, and other fomites contaminated by infected saliva. In underdeveloped areas of the world, HSV-1 antibody has an approximate 90% prevalence rate by the age of 2 years. HSV-2 is disseminated mainly by sexual contact, autoinoculation, or from mother-to-infant at birth. Both viruses can cause significant morbidity and mortality upon infection of the eye, brain, and as a disseminated infection in immunosuppressed individuals or neonates.



The various types of infections produced by HSV-1 and HSV-2 are oral herpes, herpetic keratitis, herpetic whitlow, eczema herpetoid, genital herpes, HSV proctitis, HSV meningitis, herpes encephalitis, neonate and newborn infection. Genital herpes is responsible for 10% of all genital type infections. Genital infections may be asymptomatic or produce numbers of painful lesions (erythematous base with clear vesicles on top) in the male and female genital regions. Lesions are accompanied by fever, malaise, body aches, and myalgia. The lesions usually break out once a month or in another reoccurring cycle.

### **Varicella zoster virus**

Varicella zoster (VZV) causes chicken pox and with recurrence it is the etiological agent of herpes zoster or shingles. VZD is spread through the respiratory route and by direct contact. After replication in the respiratory tract, viremia occurs, and then characteristic skin lesions appear over the entire body. In developed countries over 90% of the adults have had chicken pox and have the VZV antibody. Infections in adults are more serious than those in children and in the immunocompromised patient the infection can be fatal.

### **Human T cell leukemia virus types I and II (HTLV I and II)**

These viruses are oncogenic retroviruses (originally called the RNA tumor viruses) and have been associated with leukemias, sarcomas, and lymphomas. The members of this family are unique because of the mechanism of cell transformation and the length of latency period between infection and development of disease. They can cause cancer after a long latent period of at least 30 years. HTLV I produces adult acute T cell lymphocytic leukemia (ATLL) and tropical spastic paraparesis (an incurable neurological disease). HTLV I is spread by blood transfusion, sexual intercourse, and breast feeding. HTLV I is endemic in southern Japan. It can also be found in the Caribbean and among African Americans in the southern United States. In the United States, HTLV I transmission by intravenous drug use and blood transfusion is on the rise. HTLV II has been associated with unusual T cell malignancies in humans. Identification is detected immunologically by the presence of viral-specific antigens in blood.

### **Human herpes virus type 6 (HHV-6)**

HHV-6 was first isolated from the blood of AIDS patients. In 1988 HHV-6 was serologically associated with a common disease of children known as roseola. It spreads by close contact or respiratory means and targets T cells. At least 45% of the children are seropositive for HHV-6 by age 2. HHV-6 may also cause a mononucleosis syndrome and lymphadenopathy in AIDS patients.

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## **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

### **212. Hepatitis testing**

1. How does a virus attach to a host cell?
2. How are the completed virus particles released from the host cell?
3. What is hepatitis and how is it caused?
4. What are the two basic categories of hepatitis and how would you describe each category?

5. What is the difference between acute and chronic hepatitis?
6. What is fulminant hepatitis?
7. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

*Column A*

- \_\_\_\_(1) I Indicative of active replication of the hepatitis virus and identifies the patient as highly contagious.
- \_\_\_\_(2) I Formerly non-A, non-B.
- \_\_\_\_(3) I Chronic hepatitis and a chronic carrier state doesn't occur.
- \_\_\_\_(4) I Individuals at risk are those with multiple sex partners, drug users, homosexual men, dialysis patients, recipients of blood or blood products, and some health care workers.
- \_\_\_\_(5) I Outbreaks are generally a result of contaminated food or water supply.
- \_\_\_\_(6) I Suspected in chronic HBV carriers who experience a sudden deterioration in their clinical condition.
- \_\_\_\_(7) I This antibody response (seroconversion) occurs normally at the peak of clinical symptoms and is indicative of resolution of the disease.
- \_\_\_\_(8) I Transmitted from person-to-person through the fecal-oral route.
- \_\_\_\_(9) I Sometimes called a "viral parasite" or "defective virus" because it can only replicate in HBV-infected cells.
- \_\_\_\_(10) I Results in approximately 80% of the cases of primary hepatocellular carcinoma.
- \_\_\_\_(11) I Most children are asymptomatic, adults have nausea, vomiting, dark urine, abdominal pain, and fatigue.
- \_\_\_\_(12) I Major cause of acute hepatitis resembling hepatitis A.
- \_\_\_\_(13) I Increases the severity of hepatitis B infections and its symptoms.
- \_\_\_\_(14) I Transmitted by parenteral (injection), sexual contact, or mother-to-infant modes.
- \_\_\_\_(15) I The major cause of post-transfusion hepatitis.
- \_\_\_\_(16) I Major sources of spreading are day-care centers, crowded living conditions, and poor personnel hygiene.
- \_\_\_\_(17) I Infections are asymptomatic to symptomatic, and acute or chronic.
- \_\_\_\_(18) I Associated with an unexplained high fatality rate among pregnant women.
- \_\_\_\_(19) I Responsible for 40% of fulminant hepatitis cases.
- \_\_\_\_(20) I Titer parallels liver enzyme values as they increase and usually disappears about the time that liver studies return to normal.
- \_\_\_\_(21) I It often leads to acute disease, cirrhosis, and, occasionally, to fulminant hepatitis and primary hepatocellular carcinoma.
- \_\_\_\_(22) I Does not appear during the acute phase of the disease, rather it manifests during the convalescent period.

*Column B*

- a. Hepatitis A virus (HAV).
- b. Hepatitis B virus (HBV).
- c. Hepatitis C virus (HCV).
- d. Hepatitis delta virus (HDV).
- e. Hepatitis E virus (HEV).
- f. Anti-HAV IgG.
- g. HBsAg.
- h. HBeAg.
- i. Anti-HBs.
- j. Anti-HBe.

**213. Human Immunodeficiency Virus (HIV) testing**

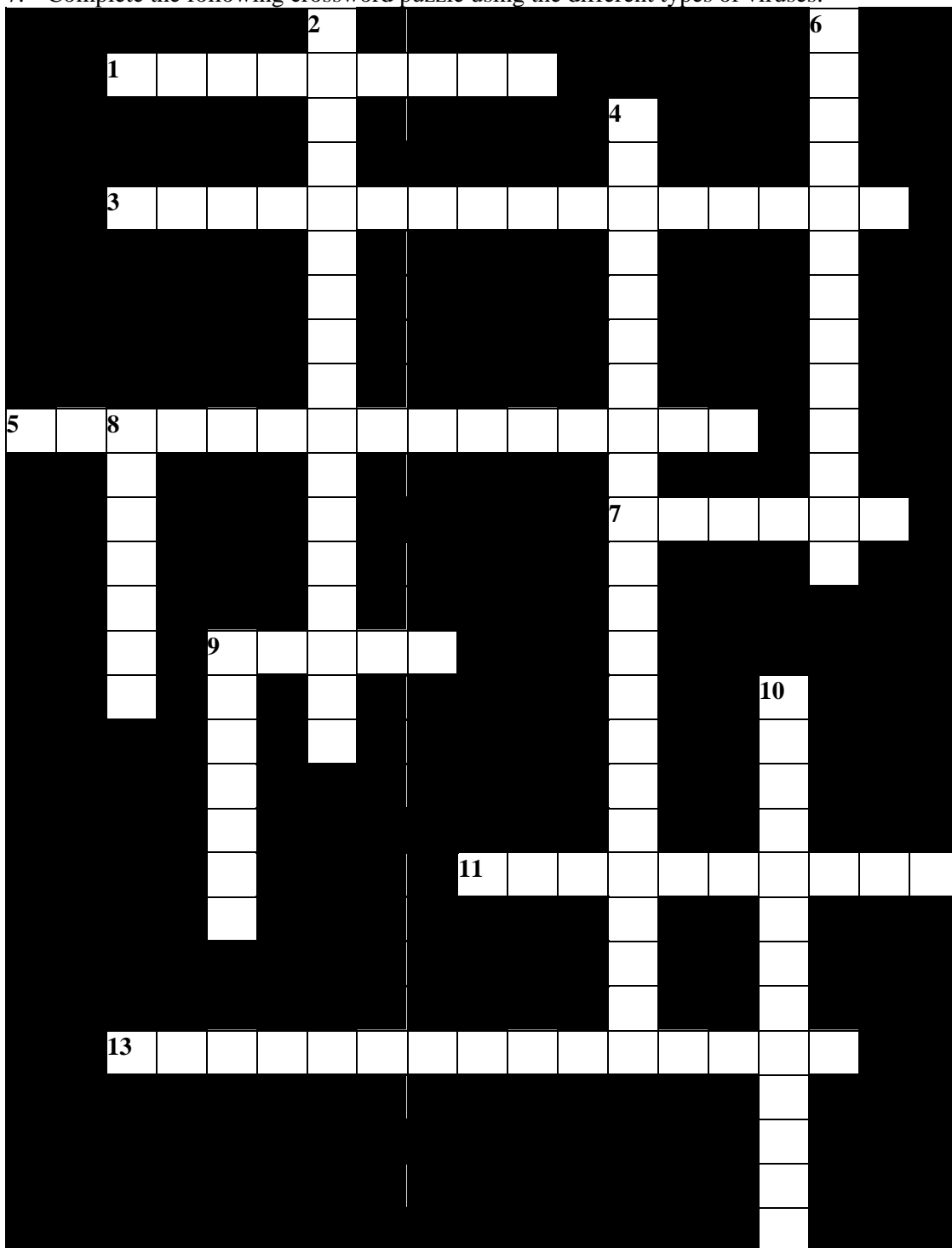
1. When was AIDS first recognized and in what group of individuals?
2. How is HIV transmitted?
3. In the United States, Europe, and Australia, what activity accounts for the majority of sexually transmitted cases?
4. In these countries, who are in the high risk groups?
5. What accounts for most of the HIV infections in Africa?
6. The time from exposure to the onset of disease depends on what factors?
7. How are HIV infections classified?
8. What are these classifications?
9. What are the symptoms of acute HIV mononucleosis?
10. What is a definition of ARC and what are the symptoms?
11. What are the Center for Disease Control and Prevention diagnostic criteria for AIDS?
12. What are the most common opportunistic infections seen in AIDS patients?
13. What are the most common types of cancers seen?

14. What is the laboratory evidence of AIDS?
15. The HIV genome consists of what three major genes and what is their purpose?
16. During the first or acute stage of the infection, what antigen can be detected using enzyme immunoassays, but only for a short time?
17. What is the most popular HIV screening procedure?
18. What are the confirmatory test and which is the most widely used?
19. Recent Center for Disease Control and Prevention criteria indicates that an HIV infection is confirmed if there is a response (antibodies) to what two antigens?
20. What test may be necessary to confirm HIV infections in asymptomatic infants?

**214. Miscellaneous viruses**

1. How is rubella spread and promoted?
2. What are the symptoms in children and in adults?
3. Who is at the gravest danger of a rubella infection?
4. What does this infection lead to in the infant?
5. What are the clinical manifestations of congenital rubella infections?
6. How is a recent rubella infection confirmed?

7. Complete the following crossword puzzle using the different types of viruses.



<i>ACROSS</i>	<i>DOWN</i>
1. Can survive on a countertops for as long as a day	2. They cause cancer after a long latent period of at least 30 years
3. Serologically associated with a common disease of children known as roseola	4. Most frequent cause of fatal acute respiratory infection in infants and young

	children with cardiorespiratory conditions
5. Causes chicken pox and, with recurrence, shingles	6. Transmitted in semen, saliva, and vaginal secretions
7. Almost always a fatal disease unless treated by vaccination	8. Severely afflicted children have multiple defects
9. Infects epithelial cells of respiratory tract and causes painful swelling of the salivary glands	9. Responsible for a chronic slowly progressive neurologic disease known as subacute sclerosing panencephalitis
11. Live vaccine (used in the United States) may produce a paralytic disease	10. Causes bronchiolitis, pneumonia, and croup in small children
13. Common viral cause of congenital defects and is a pathogen in immunocompromised patients	

### Answers to Self-Test Questions

#### 210

1. (1) c.
- (2) a.
- (3) e.
- (4) f.
- (5) g.
- (6) a.
- (7) e.
- (8) f.
- (9) b.
- (10) h.
- (11) i.
- (12) g.
- (13) e.
- (14) b.
- (15) e.
- (16) d.
- (17) a.
- (18) f.
- (19) g.
- (20) i.
- (21) h.
- (22) h.

#### 211

1. Venereal and endemic.
2. Venereal syphilis is transmitted from person-to-person through intimate contact, transfusion of fresh human blood, the placenta (congenital), fomites, or direct inoculation.
3. Due to changes in sexual practices (e.g., homosexuality, increased number of sexual partners, etc.).

4. Via the circulatory system; involves lymphocytes, plasma cells, and macrophages. It produces reagin, anticardiolipin antibodies, antitreponemal antibodies, and other immune complexes.
5. Primary, secondary, latent period, and tertiary (late) syphilis.
6. A primary lesion appears around 3 weeks after infection and diagnosis is made by darkfield examination of fluid.
7. Secondary syphilis is characterized by headache, fever, sore throat, lymphadenopathy, lesions in the anal and genital regions, and a localized or generalized rash; darkfield examination of lesions or serologic tests.
8. No signs or symptoms; nontreponemal serologic procedures may be nonreactive but treponemal procedures are reactive.
9. Tertiary syphilis may involve the cardiovascular system, central nervous system, eyes, and ears; generally, only treponemal procedures are reactive in this stage.
10. The development of an aortic aneurysm which may rupture and result in immediate death.
11. Nontreponemal and treponemal.
12. Rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL).
13. Fluorescent treponemal antibody absorption test (FTA-ABS) and microhemagglutination *Treponema pallidum* (MHA-TP).
14. Reagin.
15. The reagent is a specially formulated cardiolipin solution, containing choline chloride for inactivation of interfering complement, and charcoal particles for visible agglutination.
16. Systemic lupus erythematosus, rheumatic fever, viral or pneumococcal pneumonia, vaccinia, DPT immunizations, infectious mononucleosis, infectious hepatitis, leprosy, malaria, rheumatoid arthritis, pregnancy, and aging individuals.
17. Extract of Reiter treponemes; to absorb antibodies common to both saprophytic and pathogenic treponemes.
18. Nichols strain.

## 212

1. Through the process of phagocytosis, the viral envelope attaches to the cell membrane, or it injects or fuses its nucleic acid core into the cell.
2. Through cell lysis, by budding through the cell, or through envelopment.
3. Inflammation of the liver; can be a result of alcoholism, amebic abscesses, autoimmune disorders, hepatotoxic drugs or chemicals, and viruses.
4. Primary and secondary; Primary category primarily attacks the liver and has little direct effect on other body systems, secondary category cause liver inflammation as a consequence of systemic infection from these viruses.
5. Acute hepatitis is typically associated with jaundice and a severe infection and chronic hepatitis lasts for 6 months or more, is accompanied by hepatic inflammation and cirrhosis, and occasionally with hepatocellular carcinoma.
6. Fulminant hepatitis is a rare form of hepatitis which results in hepatic failure with a high mortality rate and is associated with hepatitis D, B, and A.
7.
  - (1) h.
  - (2) c.
  - (3) a.
  - (4) b, c.
  - (5) a.
  - (6) d.
  - (7) j.
  - (8) a, e.
  - (9) d.
  - (10) b.

- (11) a.
- (12) e.
- (13) d.
- (14) b.
- (15) c.
- (16) a.
- (17) b.
- (18) e.
- (19) d.
- (20) g.
- (21) c.
- (22) i.

**213**

1. In the late 1970s and early 1980s, it was noted that an unusual number of young homosexual men, heroin addicts, hemophiliacs, and Haitians were dying of normal benign opportunistic infections.
2. Through sexual contact, infected blood and blood products, contaminated needles or syringes, artificial insemination with infected semen, and from mother to infant during pregnancy, birth, or breast feeding.
3. Homosexual activity.
4. Homosexual and bisexual men, intravenous drug users, multi-partner sexually active persons, prostitutes, and children born to infected mothers. Before 1985 (the beginning of routine screening), blood and organ transplant recipients and hemophiliacs.
5. Heterosexual contact, specifically from infected female prostitutes to their clients, is believed to account for most of the HIV infections.
6. The viral inoculum, virulence of strain, mode of transmission, and age of the patient.
7. HIV infections have been classified according to serological results, clinical symptoms, presence of opportunistic infections or certain cancers, and CD4 lymphocyte depletion.
8. Asymptomatic, acute HIV mononucleosis, AIDS-related complex (ARC), and AIDS may be used to describe the classifications of HIV infection.
9. Acute HIV mononucleosis symptoms are fever, headache, sore throat, fatigue, body discomfort, rash, lymphadenopathy, hepatosplenomegaly, and possibly neurologic disease.
10. ARC basically encompasses patients who are chronically ill, but fall somewhere between acute HIV mononucleosis and the Center for Disease Control and Prevention's criteria for AIDS. Symptoms consist of a persistent fever, night sweats, weight loss, unexplained chronic diarrhea, eczema, psoriasis, and generalized lymphadenopathy.
11. Presence of certain opportunistic infections and cancers, HIV-related encephalopathy, HIV-induced wasting away syndrome, and laboratory evidence of infection.
12. *Pneumocystis carinii* pneumonia, toxoplasmosis, *Mycobacterium avium* complex disease, disseminated *Mycobacterium tuberculosis* infection, disseminated cytomegalovirus infection, and persistent ulcerative herpes simplex virus infection.
13. Kaposi's sarcoma, neoplasms of the skin, and B cell lymphomas.
14. Laboratory evidence is a CD4 lymphocyte count of less than 200 cells/ $\mu$ l or a CD4 T lymphocyte level of <14% of total lymphocyte count.
15. These genes are the *gag* (group-specific antigen), *pol* (polymerase), and *env* (envelope) and they encode for enzymatic and structural proteins for replication.
16. The HIV p24 (core protein) antigen.
17. ELISA.
18. Western blot, immunofluorescence assays, or ELISA with recombinant antigens; Western blot.
19. p24, gp41, or gp120/160.
20. Culture of the actual virus from blood or tissue, or PCR HIV genome amplification.



[illegible]

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

40. (210) Cold agglutinins are autoantibodies that agglutinate human red blood cells of the
  - a. M and N systems at temperatures  $<37^{\circ}\text{C}$ .
  - b. Ii and Pr systems at temperatures  $<37^{\circ}\text{C}$ .
  - c. M and N systems at temperatures  $>37^{\circ}\text{C}$ .
  - d. Ii and Pr systems at temperatures  $>37^{\circ}\text{C}$ .
41. (210) Which one of the following diseases is associated with a variety of infections, especially mycoplasma pneumonia and infectious mononucleosis?
  - a. Lymphoproliferative.
  - b. Primary cold agglutinin disease.
  - c. Paroxysmal cold hemoglobinuria.
  - d. Secondary cold agglutinin disease.
42. (210) Which one of the following illnesses does the Epstein-Barr virus (EBV) most commonly cause?
  - a. Lymphoma.
  - b. Burkitt's lymphoma.
  - c. Infectious mononucleosis.
  - d. Nasopharyngeal carcinoma.
43. (210) Infectious mononucleosis is *usually* diagnosed through serological tests that
  - a. cultivate EBV.
  - b. detect antibodies to reagin.
  - c. detect the presence of heterophil antibodies.
  - d. detect the Epstein-Barr nuclear antigen.
44. (210) In the Davidsohn differential test infectious mononucleosis heterophil antibodies are
  - a. absorbed by GPK cells and beef erythrocytes.
  - b. *not* absorbed by GPK cells and beef erythrocytes.
  - c. absorbed by GPK cells and are *not* absorbed by beef erythrocytes.
  - d. *not* absorbed by GPK cells and are absorbed by beef erythrocytes.
45. (210) Which of the following are complications from streptococcal pharyngitis and streptococcal skin infections?
  - a. Rheumatic fever and acute glomerulonephritis.
  - b. Rheumatoid arthritis and acute glomerulonephritis.
  - c. Rheumatic fever and systemic lupus erythematosus.
  - d. Rheumatoid arthritis and systemic lupus erythematosus.
46. (210) Streptococcus pyogens related rheumatic fever and acute glomerulonephritis are diagnosed by
  - a. serotyping the organism.
  - b. isolating the organism from the infected organs.
  - c. detecting antinuclear antibodies and anti-DNA in serum.
  - d. detecting antistreptolysin O, antistreptokinase, antihyaluronidase, and antideoxyribonuclease-B in serum.

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47. (210) What test is *most* reliable in detecting previous group A streptococcal infections?
- Streptozyme.
  - Antihyaluronidase test.
  - Antistreptolysin O neutralization.
  - Antideoxyribonuclease-B test (DNase B).
48. (210) Which of the following is a chronic recurrent systemic inflammatory disease that *primarily* involves the joints?
- Behçet's disease.
  - Sjögren's syndrome.
  - Rheumatoid arthritis.
  - Systemic lupus erythematosus.
49. (210) Rheumatoid arthritis procedures are unique because they
- detect C-reactive proteins.
  - detect complement activation.
  - use RF antigens to detect RF antibodies.
  - use an immunoglobulin as the antigen to detect and react with RF (immunoglobulins).
50. (210) The hallmarks of active SLE are
- increased serum complement and the presence of anti-ds-DNA.
  - diminished serum complement and the presence of anti-ds-DNA.
  - increased serum complement and the presence of antinuclear antibodies.
  - diminished serum complement and the presence of antinuclear antibodies.
51. (210) For the assay to be specific for hCG, the antibody *must* recognize the unique section of which subunit on the hCG molecule?
- $\alpha$ .
  - $\beta$ .
  - $\delta$ .
  - $\gamma$ .
52. (210) Secretion of high levels of hCG in the range of 5,000 to 6,000,000 mIU/ml *may* indicate
- eclampsia.
  - ectopic pregnancy.
  - trophoblastic tumors.
  - multiple pregnancies.
53. (211) The etiological agent of venereal syphilis is
- Treponema carateum*.
  - Treponema pallidum* ssp. *pertenue*.
  - Treponema pallidum* ssp. *pallidum*.
  - Treponema pallidum* ssp. *endemicum*.
54. (211) In the basic rapid plasma reagin, the card is placed on a slide rotator circumscribing a 3/4 inch circle for
- 5 minutes at 100 rpm.
  - 5 minutes at 180 rpm.
  - 8 minutes at 100 rpm.
  - 8 minutes at 180 rpm.

55. (211) For the CSF VDRL, the antigen is mixed with an equal volume of 10% saline to increase the
- sensitivity.
  - amount of CSF.
  - antigen dilution.
  - amount of reagent.
56. (212) What hepatitis is rare and results in hepatic failure with a high mortality rate?
- Acute.
  - Chronic.
  - Fulminant.
  - Subclinical.
57. (212) Which of the following individuals are *not* at risk for hepatitis B virus?
- Drug users.
  - Homosexual men.
  - Children at daycare centers.
  - Persons with multiple sex partners.
58. (212) Which of the following is the *major* cause of post-transfusion hepatitis?
- HAV.
  - HBV.
  - HCV.
  - HEV.
59. (213) It is *unlikely* that HIV is transmitted through
- blood.
  - saliva.
  - semen.
  - contaminated needles.
60. (213) In an HIV infection, *usually* the first two antibodies to appear are
- anti-gp41 and anti-p17.
  - anti-gp41 and anti-p24.
  - anti-gp120 and anti-p17.
  - anti-gp120 and anti-p24.
61. (213) The most popular HIV screening procedure is
- HAI.
  - EMIT.
  - FPIA.
  - ELISA.
62. (213) Which of the following is *not* an HIV confirmatory test?
- ELISA.
  - Western blot.
  - Immunofluorescence assays.
  - ELISA with recombinant antigens.
63. (213) Recent Center for Disease Control and Prevention criteria indicates that an HIV infection is confirmed if there is a response to any two of the following Western blot bands,
- p51, p55, or gp41.
  - p31, p51, or gp41.
  - p17, p31, or gp120/160.
  - p24, gp41, or gp120/160.

64. (213) Which of the following *may* be necessary to confirm HIV infection in asymptomatic infants?
- a. ELISA.
  - b. Western blot.
  - c. PCR amplification.
  - d. Immunofluorescent assays.
65. (214) What viral infection usually causes a mild exanthematous childhood disease, but has serious consequences for the neonate?
- a. Polio.
  - b. Rubella.
  - c. Measles.
  - d. Parainfluenza.
66. (214) To confirm a recent Rubella infection, perform
- a. an EIA or IFA specific for IgG.
  - b. an EIA or IFA specific for IgM.
  - c. a rapid agglutination card test for IgG.
  - d. a rapid agglutination card test for IgM.
67. (214) What virus is the *most frequent* cause of fatal acute respiratory infection in infants and young children with cardiorespiratory conditions?
- a. CMV.
  - b. RSV.
  - c. HTLV I and II.
  - d. Influenza A and B.
68. (214) What is the *most common* viral cause of congenital defects and is a pathogen in immunocompromised patients?
- a. HAV.
  - b. CMV.
  - c. RSV.
  - d. VZV.
69. (214) What virus can be fatal in the immunocompromised patient and with recurrence causes shingles?
- a. HSV.
  - b. CMV.
  - c. RSV.
  - d. VZV.

When you complete this course, please complete the student survey on the Internet at this URL: <http://www.maxwell.af.mil/au/afiadl/>. Click on Student Info and choose 9502 Survey.

## Student Notes

## Glossary

### Terms

**Antigenic**—Having the properties of an antigen, capable of illiciting an immune response.

**Antiphagocytic**—Counteracting or opposing phagocytosis.

**Antisera**—Serum that contains antibodies; it may be obtained from an animal that has been immunized either by injection of antigen into the body.

**Aplastic**—A deficient development or failure to develop.

**Asymptomatic**—Showing or causing no symptoms.

**Autolysis**—The spontaneous disintegration of tissues or of cells by the action of their own autogenous enzymes, such as occurs after death.

**Cellulitis**—Inflammation of cellular tissue; especially purulent inflammation of the loose subcutaneous tissue.

**Clone**—A strain of cells that are derived from a single cell.

**Colorimetric**—A chemical procedure in a reagent reacts with a particular chemical constituent to produce a measurable color.

**Colorimetry**—The use of color density measurement in the assaying of a given constituent.

**Cytology**—The science that deals with the function and structure of body cells.

**Dalton**—A unit of mass, being one-sixteenth of the mass of the oxygen atom.

**Genome**—The complete gene complement of an organism, contained in a set of chromosomes in eukaryotes, a single chromosome in bacteria, or a DNA or RNA molecule in viruses.

**Glomerulonephritis**—Inflammation of the capillary loops in the glomeruli of the kidney.

**Glomerulus**—A tuft or cluster; used in anatomical nomenclature as a general term to designate such a structure.

**Hemolysis**—The liberation of hemoglobin.

**Hepatotoxicity**—The quality or property of exerting a destructive or poisonous effect upon liver cells.

**Immunodeficiency**—A deficiency in immune response, either in that mediated by humoral antibody or in that mediated by immune lymphoid cells.

**Immunoglobulins**—A protein of animal origin endowed with known antibody activity.

**Immunosuppression**—The artificial prevention or diminution of the immune response, as by irradiation or by administration of antimetabolites, antilymphocyte serum, or specific antibody.

**Lipoproteins**—A combination of a lipid and protein, possessing the general properties of proteins.

**Lymphadenitis**—Inflammation of one or more lymph nodes, usually caused by a primary focus of infection elsewhere in the body.

**Lymphoid**—Resembling or pertaining to lymph or tissue of the lymphatic system.

**Lymphoma**—A general term applied to any neoplastic disorder of the lymphoid tissue, including Hodgkin's disease.

**Lyse**—To cause or produce disintegration of a compound, substance, or cell.

**Meningitis**—Inflammation of the meninges.

Meningoencephalitis–Inflammation of the brain and meninges.

Monoclonal–Cells derived from a single cell or clone.

Mucosa–A mucus membrane.

Necrosis–Death of tissue, usually as individual cells, groups of cells, or in small localized areas.

Neoplasm–A new tissue growth that is out of control.

Petechiae–Small, pinpoint hemorrhages.

Phagocyte–Any cell that ingests microorganisms or other cells and foreign particles.

Pharyngitis–Inflammation of the pharynx.

Pneumonia–Inflammation of the lungs with consolidation.

Purpura–A group of disorders characterized by skin discoloration due to hemorrhages under the skin.

Purulent–Consisting of or containing pus; associated with the formation of or caused by pus.

Pyogenic–Pus producing.

Pyretic–Having to do with fever.

Reiter's syndrome–Arthritis associated with non-bacterial urethritis or cervicitis, conjunctivitis, and mucocutaneous lesions.

Virulence–The degree of pathogenicity of a microorganism as indicated by the severity of the disease produced and its ability to invade the tissues of a host.

## **Abbreviations and Acronyms**

<b>AGN</b>	Acute glomerulonephritis
<b>AHT</b>	Antihyaluronidase test
<b>ALG</b>	Antilymphocyte globulin
<b>ALS</b>	Antilymphocyte serum
<b>ARC</b>	AIDS-related complex
<b>ATG</b>	Antithymocyte globulin
<b>ATLL</b>	Acute T cell lymphocytic leukemia
<b>ATS</b>	Antithymocyte serum
<b>CMV</b>	Cytomegalovirus
<b>CID</b>	Cytomegalic inclusion disease
<b>CMV</b>	Cytomegalovirus
<b>CID</b>	Cytomegalic inclusion disease
<b>CIEP</b>	Countercurrent immunoelectrophoresis
<b>CF</b>	Complement fixation
<b>DAT</b>	Direct antiglobulin test
<b>DNase B</b>	Antideoxyribonuclease-B B test
<b>EA</b>	Early antigen



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<b>EBNA</b>	Epstein-Barr nuclear antigen
<b>EBV</b>	Epstein-Barr virus
<b>ELISA</b>	Enzyme-linked immunoabsorbent assay
<b>EMIT</b>	Enzyme-multiplied immunoassay techniques
<b>EIA</b>	Enzyme immunoassay
<b>FACS</b>	Fluorescent active cell sorter
<b>FPIA</b>	Fluorescence polarization immunoassays
<b>FSH</b>	Follicle stimulating hormone
<b>FTA-ABS</b>	Fluorescent treponemal antibody absorption test
<b>GPk</b>	Guinea pig kidney
<b>HA</b>	Hemagglutination
<b>HAA</b>	Hepatitis-associated antigen
<b>HAI</b>	Hemagglutination inhibition
<b>hCG</b>	Human chorionic gonadotrophin
<b>HDN</b>	Hemolytic disease of the newborn
<b>HTLV I</b>	
<b>and II</b>	Human T cell leukemia virus types I and II
<b>IEP</b>	Immunoelectrophoresis
<b>IFE, IFX</b>	Immunofixation electrophoresis
<b>LA</b>	Latex agglutination
<b>LAI</b>	Latex agglutination inhibition
<b>PBS</b>	Phosphate buffered saline
<b>PHA</b>	Pallidum hemagglutination assay
<b>RA</b>	Rheumatoid arthritis
<b>RID</b>	Radial immunodiffusion
<b>RIA</b>	Radioimmunoassay
<b>RF</b>	Rheumatoid factors
<b>RF</b>	Rheumatic fever
<b>RPR</b>	Rapid plasma reagin
<b>RST</b>	Reagin screen test
<b>SLE</b>	Systemic lupus erythematosus
<b>TSH</b>	Thyroid stimulating hormone
<b>USR</b>	Serum regain test
<b>VCA</b>	Viral capsid antigen
<b>VDRL</b>	Veneral Disease Research Laboratory
<b>VZV</b>	Varicella zoster virus

## Student Notes

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