

# LAB 17: SEROLOGY: DIRECT AND INDIRECT SEROLOGIC TESTING

## DISCUSSION

### A. INTRODUCTION TO SEROLOGIC TESTING

The adaptive immune responses refer to the **ability of the body (self) to recognize specific foreign antigens (non-self)** that threaten its biological integrity. There are two major branches of the adaptive immune responses:

1. **humoral immunity:** humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.
2. **cell-mediated immunity:** Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

To understand the immune responses we must first understand what is meant by the term antigen. Technically, an **antigen** is defined as **a substance that reacts with antibody molecules and antigen receptors on lymphocytes**. An **immunogen** is **an antigen that is recognized by the body as nonself and stimulates an adaptive immune response**. For simplicity, both antigens and immunogens are usually referred to as antigens.

Chemically, **antigens are large molecular weight proteins** (including conjugated proteins such as glycoproteins, lipoproteins, and nucleoproteins) **and polysaccharides** (including lipopolysaccharides). These protein and polysaccharide antigens are found on the surfaces of viruses and cells, including microbial cells (bacteria, fungi, protozoans) and human cells.

As mentioned above, the B-lymphocytes and T-lymphocytes are the cells that carry out adaptive immune responses. The body recognizes an antigen as foreign when that antigen binds to the surfaces of B-lymphocytes and T-lymphocytes by way of antigen-specific receptors having a shape that corresponds to that of the antigen, similar to interlocking pieces of a puzzle. The antigen receptors on the surfaces of B-lymphocytes are antibody molecules called B-cell receptors or sIg; the receptors on the surfaces of T-lymphocytes are called T-cell receptors (TCRs).

The **actual portions or fragments of an antigen that react with receptors on B-lymphocytes and T-lymphocytes, as well as with free antibody molecules, are called epitopes**. The size of an epitope is generally thought to be equivalent to **5-15 amino acids or 3-4 sugar residues**. Some antigens, such as polysaccharides, usually have many epitopes, but all of the same specificity. This is because polysaccharides may be composed of hundreds of sugars with branching sugar side chains, but usually contain only one or two different sugars. As a result, most "shapes" along the polysaccharide are the same ([see Fig. 1](#)). Other antigens such as proteins usually have many epitopes of different specificities. This is because proteins are usually hundreds of amino acids long and are composed of 20 different amino acids. Certain amino acids are able to interact with other amino acids in the protein chain and this causes the protein to fold over upon itself and assume a complex three-dimensional shape. As a result, there are many different "shapes" on the protein ([see Fig. 2](#)). That is why proteins are more immunogenic than polysaccharides; they are chemically more complex.

A microbe, such as a single bacterium, has many different proteins (and polysaccharides) on its surface that collectively form its various structures, and each different protein may have many different epitopes. Therefore, **immune responses are directed against many different parts or epitopes of the same microbe**.

[Flash animation of epitopes reacting with specific sIg on B-lymphocytes.](#)

[html5 version of animation for iPad showing epitopes reacting with specific B-cell receptor on a B-lymphocytes.](#)

**In terms of infectious diseases**, the following may act as antigens:

1. **Microbial structures** (cell walls, capsules, flagella, pili, viral capsids, envelope-associated glycoproteins, etc.); and
2. **Microbial toxins**

Certain **non-infectious materials** may also act as antigens if they are recognized as "nonself" by the body. These include:

1. **Allergens** (dust, pollen, hair, foods, dander, bee venom, drugs, and other agents causing allergic reactions);

2. **Foreign tissues and cells** (from transplants and transfusions); and

3. **The body's own cells that the body fails to recognize as "normal self"** (cancer cells, infected cells, cells involved in autoimmune diseases).

**Antibodies or immunoglobulins** are specific protein configurations produced by B-lymphocytes and plasma cells **in response to a specific antigen and capable of reacting with that antigen**. Antibodies are produced in the **lymphoid tissue** and once produced, are found mainly in the **plasma** portion of the blood (the liquid fraction of the blood before clotting). **Serum** is the liquid fraction of the blood after clotting.

There are 5 classes of human antibodies: IgG, IgM, IgA, IgD, and IgE. The simplest antibodies, such as IgG, IgD, and IgE, are "Y"-shaped macromolecules called monomers composed of **four glycoprotein chains**. There are two identical **heavy chains** having a high molecular weight that varies with the class of antibody. In addition, there are two identical **light chains** of one of two varieties: kappa or gamma. The light chains have a lower molecular weight. The four glycoprotein chains are connected to one another by disulfide (S-S) bonds and noncovalent bonds ([see Fig. 3A](#)). Additional S-S bonds fold the individual glycoprotein chains into a number of distinct globular domains. The area where the top of the "Y" joins the bottom is called the hinge. This area is flexible to enable the antibody to bind to pairs of epitopes various distances apart on an antigen.

The two tips of the "Y" monomer are referred to as the **Fab portions** of the antibody ([see Fig. 3A](#)). The first 110 amino acids or first domain of both the heavy and light chain of the Fab region of the antibody **provide specificity for binding an epitope on an antigen**. The Fab portions **provide specificity for binding an epitope on an antigen**. The bottom part of the "Y" is called the **Fc portion** and this part is responsible for the **biological activity** of the antibody (see diagram of IgG; [Fig. 3A](#)). Depending on the class of antibody, biological activities of the Fc portion of antibodies include the ability to activate the complement pathway (IgG & IgM), bind to phagocytes (IgG, IgA), or bind to mast cells and basophils (IgE).

Two classes of antibodies are more complex. IgM is a pentamer ([see Fig. 3B](#)), consisting of 5 "Y"-like molecules connected at their Fc portions, and secretory IgA is a dimer consisting of 2 "Y"-like molecules ([see Fig. 3C](#)).

For more information on antigens, antibodies, and antibody production, see the following Learning Objects in your Lecture Guide:

- [Antigens; Unit 6, Section IA2](#)
- [Antibody Structure; Unit 6 Section IIA2](#)

**Serology** refers to using antigen-antibody reactions in the laboratory for **diagnostic purposes**. Its name comes from the fact that **serum, the liquid portion of the blood where antibodies are found** is used in testing. **Serologic testing** may be used in the clinical laboratory in two distinct ways:

- To identify unknown antigens** (such as microorganisms). This is called **direct serologic testing**. **Direct serologic testing uses a preparation known antibodies, called antiserum, to identify an unknown antigen** such as a microorganism.
- To detect antibodies being made against a specific antigen in the patient's serum**. This is called **indirect serologic testing**. **Indirect serologic testing** is the procedure by which **antibodies in a person's serum being made by that individual against an antigen associated with a particular disease are detected using a known antigen**.

**Antigen-antibody reactions may be detected in the laboratory by a variety of techniques**. Some of the commonly used techniques for observing in vitro antigen-antibody reactions are briefly described below.

a. **Agglutination**

Known antiserum causes bacteria or other particulate antigens to clump together or agglutinate. Molecular-sized antigens can be detected by attaching the known antibodies to larger, insoluble particles such as latex particles or red blood cells in order to make the agglutination visible to the naked eye.

b. **Precipitation**

Known antiserum is mixed with soluble test antigen and a cloudy precipitate forms at the zone of optimum antigen-antibody proportion.

c. **Complement-fixation**

Known antiserum is mixed with the test antigen and complement is added. Sheep red blood cells and hemolysins (antibodies that lyse the sheep red blood cells in the presence of free complement) are then added. If the complement is tied up in the first antigen-antibody reaction, it will not be available for the sheep red blood cell-hemolysin reaction and there will be no hemolysis. A negative test would result in hemolysis.

d. **Enzyme-linked immunosorbant assay or ELISA (also known as Enzyme immunoassay or EIA)**

Test antigens from specimens are passed through a tube (or a membrane) coated with the corresponding specific known antibodies and become trapped on the walls of the tube (or on the membrane). Known antibodies to which an enzyme has been chemically attached are then passed through the tube (or membrane) where they combine with the trapped antigens. Substrate for the attached enzyme is then added and the amount of antigen-antibody complex formed is proportional to the amount of enzyme-substrate reaction as indicated by a color change.

#### e. **Radioactive binding techniques**

Test antigens from specimens are passed through a tube coated with the corresponding specific known antibodies and become trapped on the walls of the tube. Known antibodies to which a radioactive isotope has been chemically attached are then passed through the tube where they combine with the trapped antigens. The amount of antigen-antibody complex formed is proportional to the degree of radioactivity.

#### f. **Fluorescent antibody technique**

A fluorescent dye is chemically attached to the known antibodies. When the fluorescent antibody reacts with the antigen, the antigen will fluoresce when viewed with a fluorescent microscope.

## **B. DIRECT SEROLOGIC TESTING: USING ANTIGEN-ANTIBODY REACTIONS IN THE LABORATORY TO IDENTIFY UNKNOWN ANTIGENS SUCH AS MICROORGANISMS.**

This type of serologic testing employs **known antiserum (serum containing specific known antibodies)**. The preparation of known antibodies is prepared in one of two ways: in animals or by hybridoma cells.

### **1. Preparation of known antisera in animals.**

Preparation of known antiserum in animals involves inoculating animals with specific known antigens such as a specific strain of a bacterium. After the animal's immune responses have had time to produce antibodies against that antigen, the animal is bled and the blood is allowed to clot. The resulting liquid portion of the blood is the serum and it will contain antibodies specific for the injected antigen.

However, one of the problems of using antibodies prepared in animals (by injecting the animal with a specific antigen and collecting the serum after antibodies are produced) is that up to 90% of the antibodies in the animal's serum may be antibodies the animal has made "on its own" against environmental antigens, rather than those made against the injected antigen. The development of monoclonal antibody technique has largely solved that problem.

### **2. Preparation of known antibodies by monoclonal antibody technique.**

One of the major breakthroughs in immunology occurred when monoclonal antibody technique was developed. **Monoclonal antibodies** are antibodies of a single specific type. In this technique, an animal is injected with the specific antigen ([see Fig. 4, step 1](#)) for the antibody desired. After appropriate time for antibody production, the animal's spleen is removed. The spleen is rich in plasma cells and each plasma cell produces only one specific type of antibody. However, plasma cells will not grow artificially in cell culture. Therefore, a plasma cell producing the desired antibody is fused with a myeloma cell, a cancer cell from bone marrow which will grow rapidly in cell culture, to produce a **hybridoma cell** ([see Fig. 4, step 2](#)). The hybridoma cell has the characteristics of both parent cells. It will **produce the specific antibodies like the plasma cell and will also grow readily in cell culture like the myeloma cell**. The hybridoma cells are grown artificially in huge vats where they produce large quantities of the specific antibody ([see Fig. 4, step 3](#)).

Monoclonal antibodies are now used routinely in medical research and diagnostic serology and are being used experimentally in treating certain cancers and a few other diseases.

### **3. The concept and general procedure for direct serologic testing.**

The concept and general procedure for using antigen-antibody reactions to identify unknown antigens are as follows:

- **Concept:**

This testing is based on the fact that **antigen- antibody reactions are very specific**. Antibodies usually react **only with the antigen that stimulated their production in the first place**, and are just as specific as an enzyme-substrate reaction. Because of this, one can use **known antiserum** (prepared by animal inoculation or monoclonal antibody technique as discussed above) to identify **unknown antigens such as a microorganism**.

- **General Procedure:**

A suspension of **the unknown antigen** to be identified is mixed with **known antiserum** for that antigen. One then looks for an antigen-antibody reaction.

Examples of serologic tests used to identify unknown microorganisms include the serological typing of *Shigella* and *Salmonella* (Lab 13), the Lancefield typing of beta streptococci (Lab 14), and the serological identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* (Lab 16). Serological tests used to identify antigens which are not microorganisms include blood typing, tissue typing, and pregnancy testing.

[Concept map for Lab 17, Part 1 - Introduction and direct serologic testing](#)

#### 4. Examples of direct serologic testing to identify unknown antigens

As stated above, this type of serologic testing uses **known antiserum (antibodies) to identify unknown antigens**. Four such tests will be looked at in lab today.

##### a. Serological Typing of *Shigella*

There are **four different serological subgroups of *Shigella***, each corresponding to a different species:

- subgroup A = *Shigella dysenteriae*
- subgroup B = *Shigella flexneri*
- subgroup C = *Shigella boydii*
- subgroup D = *Shigella sonnei*

Known antiserum is available for each of the 4 subgroups of *Shigella* listed above and contains antibodies against the cell wall ("O" antigens) of *Shigella*. The suspected *Shigella* (the unknown antigen) is placed in each of 4 circles on a slide and a different known antiserum (A, B, C or D) is then added to each circle. A positive antigen-antibody reaction appears as a clumping or agglutination of the *Shigella* ([see Fig. 5](#)).

##### b. Serological Typing of Streptococci

The **Clearview® Strep A Exact II Dipstick** is a qualitative serologic test for **detecting Group A Streptococcal antigen** (the unknown antigen) directly from throat swabs and is used as an aid in **diagnosing streptococcal pharyngitis** caused by *Streptococcus pyogenes* (Group A Beta Streptococci).

The test consists of a membrane strip that is precoated with **rabbit anti-Strep A antibody-red latex conjugate (known antibody with red latex particles attached) located in a pad** at the beginning of the strip. It is also precoated with **rabbit anti-Strep A antibody** (known antibody without attached red latex) that is immobilized at the test line where the test results are read ([see Fig. 6A](#)). The **red latex particles** attached to the rabbit anti-Strep A antibody is what ultimately causes the "positive" red band.

When the test strip is immersed in the extracted sample, the **Group A Streptococcal antigen** extracted from the *Streptococcus pyogenes* on the throat swab of a person with strep throat begins to move chromatographically up the membrane and **binds to the red-colored known antibody-latex conjugate in the pad** located at the beginning of the strip, forming a Strep A antigen-antibody complex ([see Fig. 6B](#)). This Strep A antigen-antibody complex continues to move up the membrane to the test line region where the immobilized rabbit anti-Strep A antibodies are located.

**If Group A Streptococcal antigen is present in the throat swab, a red-colored sandwich of antibody/Strep A antigen/red latex conjugate antibody forms in the test line region** of the strip ([see Fig. 6C](#)). **The red color at the control line region appears when enough reagent has reached the control area and indicates that the test is finished.** As a result, **a positive test for Group A Strep antigen appears as a red band in the test result area and a red band in the control area** ([see Fig. 6C](#)).

If there is no Group A Streptococcal antigen present in the throat swab **no red band appears in the test result region** of the strip and **a single red band** appears in the control line region, **indicating a negative test for Group A Strep antigen** ([see Fig. 6D](#)).

[Flash Animation showing serologic identification of Group A Streptococci, part-1.](#)

[http5 version of animation for iPad showing serologic identification of Group A Streptococci, part-1.](#)

[Flash Animation showing serologic identification of](#)

[Group A Streptococci, part-2.](#)

[http5 version of animation for iPad showing serologic identification of Group A Streptococci, part-2.](#)

### c. Serological Testing to Diagnose Pregnancy

The **Alere® hCG Dipstick** is a qualitative serologic test for **detecting early pregnancy**. The hormone **human chorionic gonadotropin (hCG)**, produced by the placenta, appears in the serum and urine of pregnant females. The hCG is composed of two subunits - alpha and beta. The Alere® hCG Dipstick is a one step pregnancy test that detects levels of hCG as low as 25 mIU/ml. **Human chorionic gonadotropin (hCG)**, the unknown antigen for which one is testing, is identified in the urine by using **known mouse monoclonal antibodies against the beta subunit of hCG bound to colloidal gold, which is red in color. It also uses known goat polyclonal antibodies against the alpha subunit of hCG which is bound to the test result region of the dipstick.**

Like the Strep A test mentioned above, this test uses a color immunochromatographic assay to detect the antigen-antibody reaction. The test consists of a membrane strip that is precoated with **known mouse anti- beta hCG antibody-colloidal gold conjugate (known antibody with red colloidal gold particles attached) located in a pad** at the beginning of the strip. It is also precoated with **known goat anti-alpha hCG antibody** (known antibody without attached red colloidal gold) that is immobilized at the test line where the test results are read ([see Fig. 7B1](#)). The red colloidal gold particles attached to the mouse anti-alpha hCG antibody is what ultimately causes the “positive” red band.

When the test strip is immersed in the urine sample, the **hCG** begins to move chromatographically up the membrane and **binds to the red-colored known anti-beta hCG antibody-gold conjugate in the pad** located at the beginning of the strip, forming a hCG antigen-antibody complex ([see Fig. 7B2](#)). This hCG antigen-antibody complex continues to move up the membrane to the test line region where the immobilized known goat anti-beta hCG antibodies are bound.

**If hCG is present in the urine, a red-colored sandwich of anti-beta antibody/hCG antigen/red gold conjugate anti-alpha antibody forms in the test line region** of the strip ([see Fig. 7B3](#)). **The red color at the control line region appears when enough reagent has reached the control area and indicates that the test is finished.** As a result, **a positive test for hCG antigen appears as a red band in the test result area and a red band in the control area** ([see Fig. 7B3](#)).

If there is **no detectable hCG antigen** present in the urine **no red band appears in the test result region** of the strip and **a single red band** appears in the control line region, **indicating a negative test for hCG antigen** ([see Fig. 7B4](#)).

[Flash Animation showing serologic identification of hCG, part-1.](#)

[http5 version of animation for iPad showing serologic identification of hCG, part-1.](#)

[Flash Animation showing serologic identification of hCG, part-2.](#)

[http5 version of animation for iPad showing serologic identification of hCG, part-2.](#)

### d. Identification of Microorganisms Using the Direct Fluorescent Antibody Technique

Certain **fluorescent dyes** can be chemically **attached to the known antibody molecules** in antiserum. The known fluorescent antibody is then mixed with the unknown antigen, such as a microorganism, fixed to a slide. After washing, to remove any fluorescent antibody not bound to the antigen, the slide is viewed with a **fluorescent microscope**.

If the fluorescent antibody reacted with the unknown antigen, the antigen will glow or fluoresce under the fluorescent microscope. If the antibody did not react with the antigen, the antibodies will be washed off the slide and the antigen will not fluoresce.



[Flash Animation showing a positive direct fluorescent antibody test.](#)

[http5 version of animation for iPad showing a positive direct fluorescent antibody test.](#)

[Flash Animation showing a negative direct fluorescent antibody test.](#)

[http5 version of animation for iPad showing a negative direct fluorescent antibody test.](#)

For example, in the direct fluorescent **antibody test for *Neisseria gonorrhoeae***, mentioned briefly in lab 16, the unknown antigen, suspected *Neisseria gonorrhoeae*, is fixed to a microscope slide. Known fluorescent antibodies made against *N. gonorrhoeae* are then added ([see Fig. 8, step 1](#)) and the slide is then washed to remove any fluorescent antibody not bound to the antigen. The slide is then viewed under a fluorescent microscope.

**If the unknown antigen is *Neisseria gonorrhoeae*, the known antibodies against *N. gonorrhoeae* with attached fluorescent dye will bind to the bacterium and will not wash off. The bacteria will fluoresce when viewed under a fluorescent microscope** ([see Fig. 8, step 2](#) and [Fig. 10](#)). If the unknown antigen is not *N. gonorrhoeae*, the known fluorescent antibodies against will wash off the slide and the bacteria will not fluoresce when viewed under a fluorescent microscope.

Many bacteria, viruses, and fungi can be identified using this technique.

[Concept map for Lab 17, Part 1 - Introduction and direct serologic testing](#)

[Medscape](#) articles on infections associated with organisms mentioned in this lab exercise. Registration to access this website is free.

- [Salmonella species](#)
- [Shigella species](#)
- [Streptococcus pyogenes](#)
- [Neisseria gonorrhoeae](#)

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## C. INDIRECT SEROLOGIC TESTING: USING ANTIGEN-ANTIBODY REACTIONS IN THE LABORATORY TO INDIRECTLY DIAGNOSE DISEASE BY DETECTING ANTIBODIES IN A PERSON'S SERUM PRODUCED AGAINST A DISEASE ANTIGEN

**Indirect serologic testing** is the procedure whereby **antibodies in a person's serum** being made by that individual against an antigen associated with a particular disease are detected using a **known antigen**.

### 1. The concept and general procedure for indirect serologic testing.

The concept and general procedure for this type of serological testing are as follows:

- **Concept:**

This type of testing is based on the fact that **antibodies are only produced in response to a specific antigen**. In other words, a person will not be producing antibodies against a disease antigen unless that antigen is in the body stimulating antibody production.

- **General Procedure:**

A sample of the **patient's serum** (the liquid portion of the blood after clotting and containing antibodies against the disease antigen if the person has or has had the disease) is mixed with the **known antigen** for that suspected disease. One then looks for an antigen-antibody reaction.

Examples of serologic tests to diagnose disease by the detection of antibodies in the patient's serum

include the various serological tests for syphilis or STS (such as the RPR, the VDRL, and the FTA-ABS tests), the tests for infectious mononucleosis, the tests for the Human Immunodeficiency Virus (HIV), the tests for systemic lupus erythematosus, and tests for variety of other viral infections.

## 2. Qualitative and quantitative serologic tests.

Indirect serologic tests may be qualitative or quantitative. A **qualitative** test only detects the presence or absence of specific antibodies in the patient's serum and is often used for screening purposes. A **quantitative** test gives the titer or amount of that antibody in the serum. **Titer indicates how far you can dilute the patient's serum and still have it contain enough antibodies to give a detectable antigen-antibody reaction.** In other words, the more antibodies being produced by the body, the more you can dilute the person's serum and still see a reaction. Quantitative serological tests are often used to follow the progress of a disease by looking for a rise and subsequent drop in antibody titer.

[Concept map for Lab 17, Part 2 - Introduction and indirect serologic testing](#)

## 3. Examples of indirect serologic tests to detect antibodies in the patient's serum

### a. The RPR Test for Syphilis

Syphilis is a sexually transmitted disease caused by the spirochete *Treponema pallidum*. The **RPR** (Rapid Plasma Reagin) Card® test is a **presumptive serologic screening test** for syphilis. The serum of a person with syphilis contains a **non-specific anti-lipid antibody** (traditionally termed **reagin**), which is not found in normal serum. The exact nature of the anti-lipid (reagin) antibody is not known but it is thought that a syphilis infection instigates the breakdown of the patient's own tissue cells. Fatty substances which are released then combine with protein from *Treponema pallidum* to form an antigen which stimulates the body to produce **antibodies against both the body's tissue lipids (non-specific or non-treponemal) as well as the *T. pallidum* protein (specific or treponemal).** The RPR Card® test detects the nonspecific antilipid antibody and is referred to as a **non-treponemal test for syphilis.**

- [scanning electron micrograph of the spirochete \*Treponema pallidum\*](#); courtesy of CDC.

It must be remembered that tests for the presence of these nonspecific antilipid antibodies are meant as a presumptive screening test for syphilis. Similar reagin-like antibodies may also be present as a result of other diseases such as malaria, leprosy, infectious mononucleosis, systemic lupus erythematosus, viral pneumonia, measles, and collagen diseases and may give biologic false-positive results (BFP). Confirming tests should be made for the presence of specific antibodies against the *T. pallidum* itself. The confirming test for syphilis is the **FTA-ABS** test discussed below. Any serologic test for syphilis is referred to commonly as an **STS** (Serological Test for Syphilis).

The **known RPR antigen** consists of **cardiolipin, lecithin, and cholesterol bound to charcoal particles** in order to make the reaction visible to the naked eye. If the patient has syphilis, the antilipid antibodies in his or her serum will cross-react with the known RPR lipid antigens giving a visible clumping of the charcoal particles ([see Fig. 1](#)).

We will do a **quantitative** RPR Card® test today in lab. Keep in mind that a quantitative test allows one to determine the **titer** or amount of a certain antibody in the serum. In this test, a constant amount of RPR antigen is added to dilutions of the patient's serum. **The most dilute sample of the patient's serum still containing enough antibodies to give a visible antigen-antibody reaction is reported as the titer.**

### b. Serologic Tests for Infectious Mononucleosis

During the course of infectious mononucleosis, caused by the Epstein-Barr virus (EBV), the body produces nonspecific **heterophile antibodies** which are not found in normal serum. As it turns out, these **heterophile antibodies will cross react with glycoprotein antigens found on the surface of red blood cells (RBCs) of various animals**, including horses, sheep, and cows, causing the RBCs to agglutinate. These cross-reacting glycoprotein antigens are often called **Paul-Bunnell antigens** after their discoverers.

The infectious mononucleosis serologic test demonstrated today is a rapid **qualitative** test for infectious mononucleosis that uses **Paul-Bunnell antigens adsorbed to microscopic white latex particles as the "known antigen."** These antigens bind specifically to the heterophile antibodies found in the serum of people with infectious mononucleosis **causing the latex particles to clump or agglutinate** ([see Fig. 2](#)). Quantitative tests may then be done to determine the titer of heterophile antibodies and follow the progress of the disease.

### c. Serologic Tests for Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus or SLE is a systemic autoimmune disease. Immune complexes become deposited between the dermis and the epidermis, and in joints, blood vessels, glomeruli of the kidneys, and the central nervous system. It is four times more common in women than in men. In SLE, autoantibodies are made against components of DNA. This test is specific for the serum **anti-deoxyribonucleoprotein antibodies** associated with SLE. The **known antigen is deoxyribonucleoprotein adsorbed to latex particles** to make the reaction more visible to the eye ([see Fig. 3](#)). This is a **qualitative** test used to screen for the presence of the disease and to monitor its course.

### d. Detecting Antibody Using the Indirect Fluorescent Antibody Technique: The FTA-ABS test for syphilis

The **indirect** fluorescent antibody technique involves three different reagents:

- a. The **patient's serum** (containing antibodies against the disease antigen if the disease is present)
- b. **Known antigen** for the suspected disease
- c. **Fluorescent anti-human gamma globulin antibodies** (antibodies made in another animal against the Fc portion of human antibodies ([see Fig. 9](#)) by injecting an animal with human serum. A fluorescent dye is then chemically attached to the anti-human gamma globulin (anti-HGG) antibodies.

The **FTA-ABS test** (Fluorescent Treponemal Antibody Absorption Test) **for syphilis** is an example of an indirect fluorescent antibody procedure. This is a **confirming test** for syphilis since it tests specifically for **antibodies in the patient's serum made in response to the syphilis spirochete, *Treponema pallidum***.

In this test, killed *T. pallidum*, (the known antigen), is fixed on a slide ([see Fig 4, step 1](#)). The patient's serum is added to the slide. If the patient has syphilis, antibodies against the *T. pallidum* will react with the antigen on the slide ([Fig. 4, step 2](#)). The slide is then washed to remove any antibodies not bound to the spirochete.

To make this reaction visible, a second animal-derived antibody made against human antibodies and labelled with a fluorescent dye (fluorescent anti-human gamma globulin) is added. These fluorescent anti-HGG antibodies react with the patient's antibodies which have reacted with the *T. pallidum* on the slide ([Fig. 4, step 3](#)). The slide is washed to remove any unbound fluorescent anti-HGG antibodies and observed with a fluorescent microscope. If the spirochetes glow or fluoresce ([see Fig. 5](#)), the patient has made antibodies against *T. pallidum* and has syphilis.

[Flash animation of the FTA-ABS test for syphilis.](#)

[http5 version of animation for iPad showing the FTA-ABS test for syphilis.](#)

Another example of the indirect fluorescent antibody test is the test for **antibodies against the measles virus**. Inactivated measles virus-infected cells (the known antigen) are fixed to a microscope slide. The patient's serum is then added. If the person has measles, antibodies of the isotype IgG will be made against the measles virus and will bind to viral epitopes on the known measles virus-infected cells. After washing the slide to remove any unbound IgG, fluorescent antihuman IgG is added. The fluorescent antihuman IgG then binds to the patient's IgG that is bound to the infected cells. When viewed with a fluorescent microscope, [the infected cells will fluoresce green](#).

### e. The EIA and Western Blot serologic tests for antibodies against the Human Immunodeficiency Virus (HIV)

In the case of the current HIV antibody tests, the **patient's serum** is mixed with **various HIV antigens produced by recombinant DNA technology**. If the person is seropositive (has repeated positive antigen-antibody tests), then HIV must be in that person's body stimulating antibody production. In other words, the person must be infected with HIV. The two most common tests currently used to detect antibodies against HIV are the **enzyme immunoassay or EIA** (also known as the enzyme-linked immunosorbent assay or ELISA) and the **Western blot or WB**. A person is considered to be seropositive for HIV infection only after an EIA screening test is repeatedly reactive and another test such as the WB has been performed to confirm the results.



The **EIA** is less expensive, faster, and technically less complicated than the WB and is the procedure initially done as a screening test for HIV infection. The various EIA tests give a spectrophotometric reading of the amount of antibody binding to known HIV antigens.

The EIA test kit **contains plastic wells to which various HIV antigens have been adsorbed** (see Fig. 6, step 1). The **patient's serum** is added to the wells and any antibodies present in the serum against HIV antigens will bind to the corresponding antigens in the wells (Fig. 6, step 2). The wells are then washed to remove all antibodies in the serum other than those bound to HIV antigens. **Enzyme-linked anti-human gamma globulin (anti HGG) antibodies** are then added to the wells. These antibodies, made in another animal against the Fc portion of human antibodies by injecting the animal with human serum, have an enzyme chemically attached. They react with the human antibodies bound to the known HIV antigens (Fig. 6, step 3). The wells are then washed to remove any anti-HGG that has not bound to serum antibodies. A **substrate specific for the enzyme** is then added and the resulting enzyme-substrate reaction causes a **color change** in the wells (Fig. 6, step 4). If there are no antibodies in the patient's serum against HIV, there will be nothing for the enzyme-linked anti-HGG to bind to and it will be washed from the wells. When the substrate is added, there will be no enzyme present in the wells to give a color change.

[Flash animation of the EIA test for antibodies against HIV.](#)

[http5 version of animation for iPad showing the EIA test for antibodies against HIV.](#)

If the initial EIA is reactive it is **automatically repeated** to reduce the possibility that technical laboratory error caused the reactive result. If the EIA is still reactive, it is then **confirmed by the Western blot test**.

The **Western blot WB** is the test most commonly used as a confirming test if the EIA is repeatedly positive. The WB is technically more complex to perform and interpret, is more time consuming, and is more expensive than the EIAs.

With the WB, the various protein and glycoprotein antigens from HIV are separated according to their molecular weight by gel electrophoresis (a procedure that separates charged proteins in a gel by applying an electric field). Once separated, the various HIV antigens are transferred to a nitrocellulose strip (see Fig. 7, step 1 and Fig. 7, step 2). The patient's serum is then incubated with the strip and any HIV antibodies that are present will bind to the corresponding known HIV antigens on the strip (Fig. 7, step 3). **Enzyme-linked anti-human gamma globulin (anti HGG) antibodies** are then added to the strip. These antibodies, made in another animal against the Fc portion of human antibodies by injecting the animal with human serum, have an enzyme chemically attached. They react with the human antibodies bound to the known HIV antigens (Fig. 7, step 4). The strip is then washed to remove any anti-HGG that has not bound to serum antibodies. A **substrate specific for the enzyme** is then added and the resulting enzyme-substrate reaction causes a **color change** on the strip (Fig. 7, step 5). If there are no antibodies in the patient's serum against HIV, there will be nothing for the enzyme-linked anti-HGG to bind to and it will be washed from the strip. When the substrate is added, there will be no enzyme present on the strip to give a color change.

[Flash animation of the WB test for antibodies against HIV.](#)

[http5 version of animation for iPad showing the WB test for antibodies against HIV.](#)

It should be mentioned that all serologic tests are capable of giving occasional **false-positive and false-negative results**. The most common cause of a false-negative HIV antibody test is when a person **has been only recently infected with HIV and his or her body has not yet made sufficient quantities of antibodies to give a visible positive serologic test**. It generally takes between 2 weeks and 3 months after a person is initially infected with HIV to convert to a positive HIV antibody test.

[Concept map for Lab 17, Part 2 - Introduction and indirect serologic testing](#)

A number of commercial rapid HIV tests have also been improved for detecting antibody against HIV. They include:

- OraQuick Advance HIV1/2®: uses either a finger-stick blood specimen or an oral specimen.
- Uni-Gold Recombigen®: uses either a finger-stick or whole blood specimen.
- Reveal G2®: Uses serum or plasma.

- Multispot HIV-1/HIV-2®: Uses serum or Plasma.

For more information on HIV and AIDS, see the following Learning Objects in your Lecture Guide:

- [Unit 4, Section IVF3: The Life Cycle of HIV](#)

[Medscape](#) articles on infections associated with organisms/diseases mentioned in this lab exercise. Registration to access this website is free.

- [Syphilis](#)
- [Infectious mononucleosis](#)
- [SLE](#)
- [HIV/AIDS](#)

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## PROCEDURE

### PROCEDURE FOR DIRECT SEROLOGIC TESTING TO DETECT UNKNOWN ANTIGENS

#### A. Serologic Typing of *Shigella*

1. Using a wax marker, draw **two circles** (about the size of a nickel) on each of two clean glass slides. Label the circles A, B, C, and D.
2. Add **one drop** of the **suspected *Shigella*** (unknown antigen) to each circle. (The *Shigella* has been treated with formalin to make it noninfectious but still antigenic.)
3. Now add **one drop** of known *Shigella* subgroup **A** antiserum to the "A" circle, **one drop** of known *Shigella* subgroup **B** antiserum to the "B" circle, **one drop** of known *Shigella* subgroup **C** antiserum to the "C" circle, and **one drop** of known *Shigella* subgroup **D** antiserum to the "D" circle.
4. **Rotate** the slide carefully for **30-60 seconds**.  
[Agglutination](#) of the bacteria, indicates a positive reaction.  
[No agglutination](#) is negative.
5. Dispose of all pipettes and slides in the disinfectant container.

#### B. Serologic Typing of Streptococci: The Clearview® Strep A Exact II Dipstick Test

1. Add **4 drops of Extraction Reagent #1** to the extraction tube. This reagent contains 2M sodium nitrite and should be pink to purple in color.
2. Add **4 drops of Extraction Reagent #2** to the extraction tube. This reagent contains 0.3M acetic acid. The solution must turn yellow in color.
3. Place the **throat swab in the extraction tube and roll it with a circular motion inside the tube**. Let stand for at least **1 minute**.
4. Squeeze the swab firmly against the extraction tube to expel as much liquid as possible from the swab and discard the swab in the biowaste container.
5. **Immerse the test strip into the extraction tube** with the arrows pointing toward the extracted sample solution. Leave the strip in the tube and start timing.
6. **Read results in 5 minutes. A red band in the control region and a red band in the test region indicates a positive test (See Fig. 6C). A single red band in the control region only indicates a negative test (See Fig. 6D).** No colored band in the control region indicates an invalid test.

#### C. Serologic Testing to Detect Pregnancy: The Alere hCG-Dipstick®

1. Dip the hCG dipstick into the urine **up to the maximum line on the strip for 5 seconds**.
2. Place the test dipstick on a flat, non-absorbant surface and **read the results at 3-4 minutes**. Do not interpret after the appropriate read time.
3. If hCG is present in the urine at a concentration of 25mIU/ml or greater, a **positive test, a pink-to-red Test line will appear along with a red Control line** in the Result Window ([see Fig. 7B3](#)). If hCG is absent or present at very low levels, a **negative test, only a red Control line appears** in the Result Window ([see Fig. 7B4](#)).

#### D. The Direct Fluorescent Antibody Technique

Observe the demonstration of a [positive direct fluorescent antibody test for \*Neisseria gonorrhoeae\*](#).

### PROCEDURE FOR INDIRECT SEROLOGIC TESTING TO DETECT ANTIBODIES IN THE PATIENT'S SERUM

#### A. The RPR® Card Test for Syphilis (demonstration)

1. Label 6 test tubes as follows: 1:1, 1:2, 1:4, 1:8, 1:16, and 1:32.
2. Using a 1.0 ml pipette, add **0.5 ml of 0.9% saline solution** into tubes **1:2, 1:4, 1:8, 1:16, and 1:32**.
3. Add **0.5 ml of the patient's serum** to the **1:1** tube (undiluted serum).
4. Add another **0.5 ml of serum** to the saline in the **1:2** tube and mix. Remove **0.5 ml from the 1:2 tube** and add it to the **1:4** tube and mix. Remove **0.5 ml from the 1:4 tube**, add to the **1:8** tube and mix. Remove **0.5 ml from the 1:8 tube**, add to the **1:16** tube and mix. Remove **0.5 ml from the 1:16 tube**, add to the **1:32** tube and mix. Remove **0.5 ml from the 1:32 tube** and **discard**. The dilution of the serum is summarized in [Fig. 8](#).
5. Using the capillary pipettes provided with the kit, add **a drop of each serum dilution** to separate circles of the RPR card. Spread the serum over the entire inner surface of the circle with the tip of the pipette, using a new pipette for each serum dilution.
6. Using the RPR antigen dispenser, add **a drop of known RPR antigen** to each circle. Do not let the needle of the dispenser touch the serum. Using disposable stirrers, mix the known RPR antigen with the serum in each circle.
7. Place the slide on a shaker and **rotate for a maximum of 4 minutes**.
8. Read the results as follows:
  - A definite [clumping](#) of the charcoal particles is reported as **reactive (R)**.
  - [No clumping](#) is reported as **non-reactive (N)**.

The greatest serum dilution that produces a **reactive** result is the **titer**. For example, if the dilutions turned out as follows, the titer would be reported as 1:4 or 4 dils.

<b>1:1</b>	<b>1:2</b>	<b>1:4</b>	<b>1:8</b>	<b>1:16</b>	<b>1:32</b>
<b>R</b>	<b>R</b>	<b>R</b>	<b>N</b>	<b>N</b>	<b>N</b>

#### B. The Serologic Tests for Infectious Mononucleosis (demonstration)

1. Place **one drop of each of the patient's serum** in circles on the test slide.
2. Add **one drop of the treated latex particles containing Paul-Bunnell antigens on their surface** (the known antigen) to each circle and mix with disposable applicator sticks.
3. **Rock the card gently for 3 minutes**, and observe for agglutination of the latex particles. Agglutination indicates the presence of **heterophile antibodies** ([see Fig. 2](#)).

#### C. The Serologic Tests for Systemic Lupus Erythematosus (SLE) (demonstration)

1. Add **one drop of each of the patient's serum** to separate circles on the test slide.
2. Add **one drop of the Latex-Deoxyribonucleoprotein reagent** (the known antigen, deoxyribonucleoprotein adsorbed to latex particles) to each serum sample and mix with disposable applicator sticks.
3. **Rock the slide gently for 3 minutes** and look for agglutination of the latex particles. Agglutination indicates the presence of **antinuclear antibodies** associated with SLE ([see Fig. 3](#)).

#### D. The FTA-ABS Test for Syphilis (Indirect Fluorescent Antibody Technique)

Observe the 35mm slide of a positive FTA-ABS test ([see Fig. 5](#)).

#### E. The EIA and WB Tests for HIV Antibodies

Observe the illustrations of the [EIA](#) and the [WB](#) tests for antibodies against HIV.

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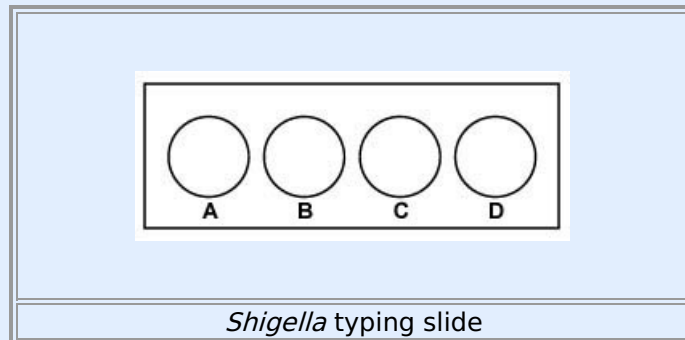
### RESULTS

#### RESULTS FOR DIRECT SEROLOGIC TESTING TO DETECT UNKNOWN ANTIGENS

##### A. Serologic Typing of *Shigella*

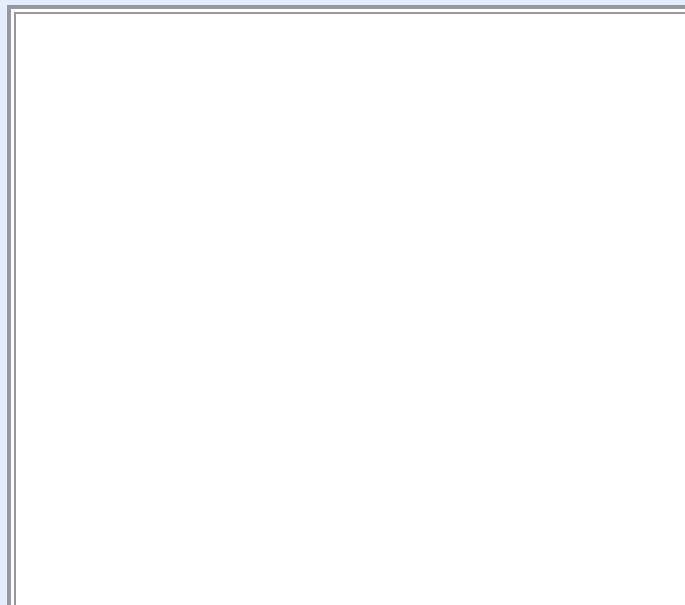
Make a drawing of your results.

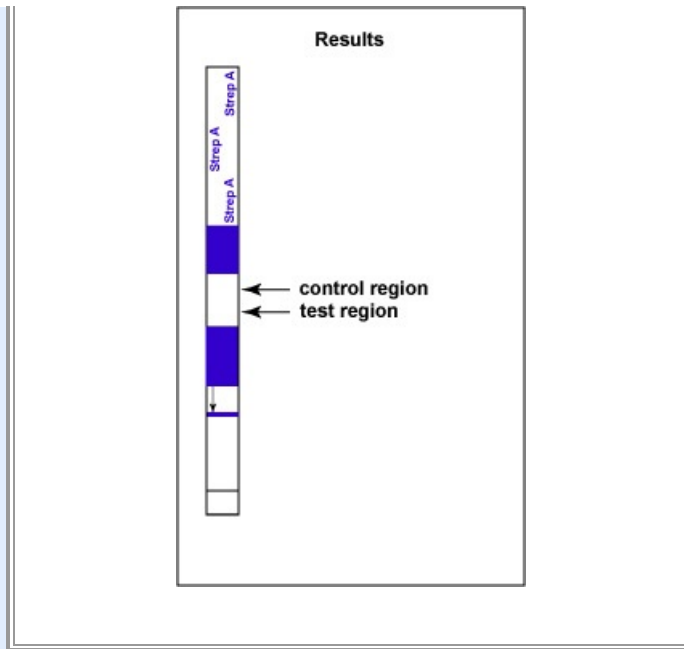
- [Agglutination](#) of bacteria is positive.
- [No agglutination](#) of bacteria is negative.



##### B. Serologic Typing of Streptococci: Clearview® Strep A Exact II Dipstick

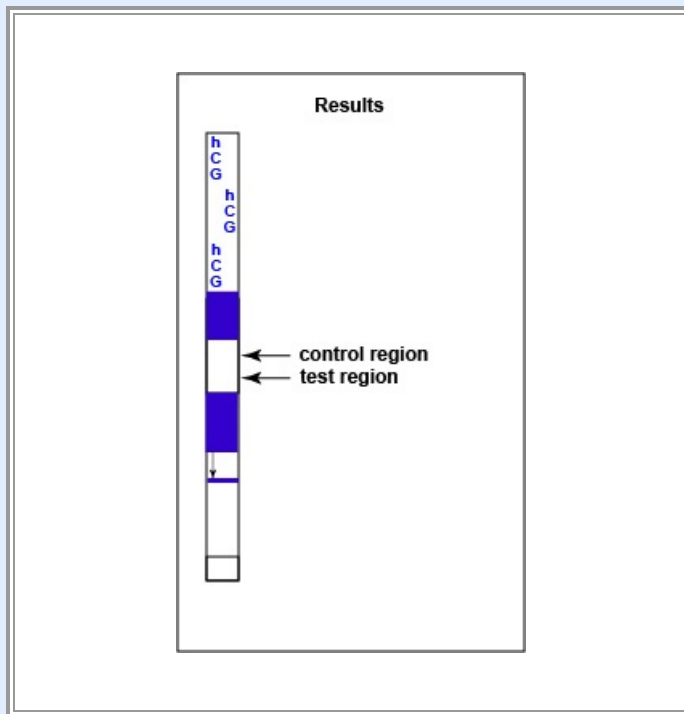
Make a drawing of your results.





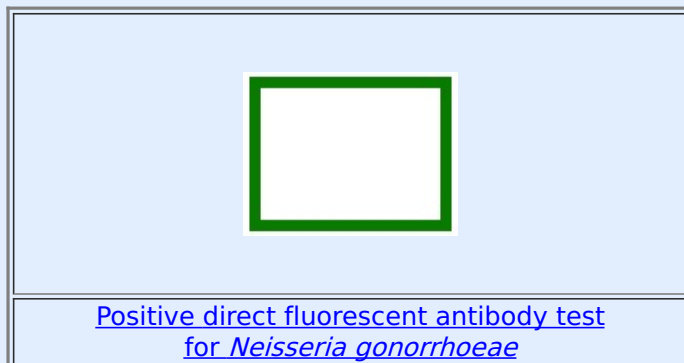
**C. Serologic Testing to Diagnose Pregnancy: Alere® hCG Dipstick**

Make a drawing of a positive test for pregnancy.



**D. The Direct Fluorescent Antibody Technique**

Make a drawing and describe a positive direct fluorescent antibody test.





### A. RPR Card® Test for Syphilis (Quantitative)

Detects nontreponemal antilipid antibodies (reagin)

Record your results in the table.

Dilution	Result
1:1	
1:2	
1:4	
1:8	
1:16	
1:32	
Titer	

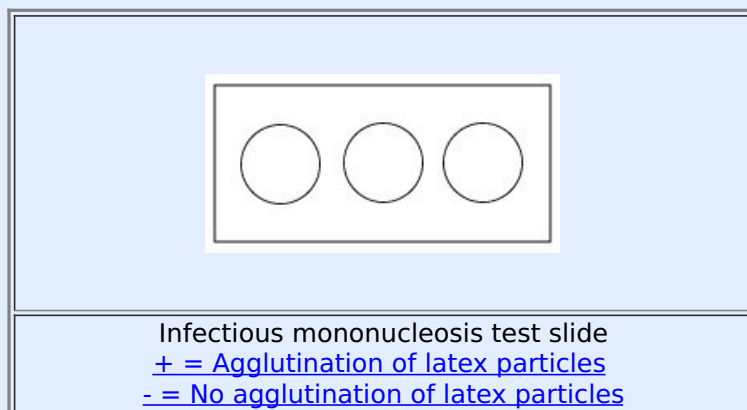
R = reactive (distinct clumps)

N = nonreactive (no clumps)

### B. MONO-TEST for Infectious Mononucleosis (Qualitative)

Detects heterophile antibodies.

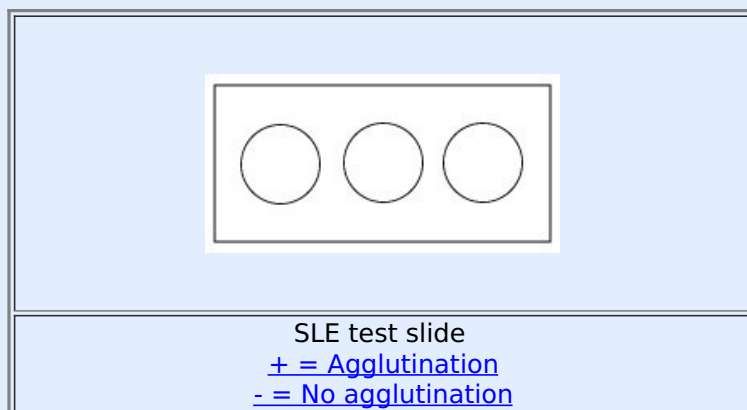
Draw the results of a positive and a negative test.



### C. Serologic test for SLE (Qualitative)

Detects anti-deoxyribonucleoprotein antibodies.

Draw the results of a positive and a negative test.



### D. FTA-ABS Test for Syphilis (Confirming)

Detects antibodies against *Treponema pallidum*

Draw the results of a positive FTA-ABS test.



[Positive FTA-ABS test for syphilis  
\(fluorescent spirochetes\)](#)

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## PERFORMANCE OBJECTIVES FOR LAB 17

After completing this lab, the student will be able to perform the following objectives:

### A. INTRODUCTION TO SEROLOGICAL TESTING

1. Define serology.
2. Define antigen and state what may act as an antigen.
3. Define antibody and state where they are primarily found in the body.
4. Define direct serologic testing and indirect serologic testing.

### B. DIRECT SEROLOGIC TESTING: USING ANTIGEN-ANTIBODY REACTIONS IN THE LAB TO IDENTIFY UNKNOWN ANTIGENS SUCH AS MICROORGANISMS

#### Discussion

1. Define antiserum.
2. Describe two ways of producing known antiserum.
3. Describe the concept and general procedure for using serologic testing to identify unknown antigens (direct serologic testing).
4. Describe how to determine serologically whether an organism is a subgroup A, B, C, or D *Shigella*.
5. Describe how to serologically identify Lancefield group A *Streptococcus* causing pharyngitis using the Rapid Response Strep A Test.
6. Describe how to diagnose pregnancy serologically using the QuickVue+® One-Step hCG-Combo Test.
7. Briefly describe the direct fluorescent antibody technique.

#### Results

1. Correctly interpret the results of the following serological tests:
  - a. serological typing of *Shigella*
  - b. serological identification of Group A streptococcal antigen using the Rapid Response Strep A Test.
  - c. serological testing for pregnancy using the QuickVue+® One-Step hCG-Combo Test
  - d. a direct fluorescent antibody test

### C. INDIRECT SEROLOGIC TESTING: USING ANTIGEN-ANTIBODY REACTIONS IN THE LAB TO DETECT ANTIBODIES IN THE PATIENT'S SERUM

#### Discussion

1. State the principle and the general procedure behind indirect serologic testing.
2. State the difference between a qualitative serological test and a quantitative serological test.
3. Define titer.
4. State what disease the RPR and the FTA-ABS procedures test for. Indicate which of these is a presumptive test, which is a confirming test, and why.
5. State the significance of non-treponemal anti-lipid (reagin) antibodies in serological testing.
6. State the significance of heterophile antibodies in serological testing.
7. State the significance of anti-deoxyribonucleoprotein antibodies in serological testing.
8. Briefly describe the indirect fluorescent antibody technique.
9. Briefly describe the EIA test for HIV antibodies and state the significance of a positive HIV antibody test.
10. State the most common reason for a false-negative HIV antibody test.

### **Results**

1. Interpret the results of the following serological tests:
  - a. serologic test for infectious mononucleosis
  - b. serologic test for SLE
  - c. FTA-ABS test
2. Determine the titer of a quantitative RPR Card® test.

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### **SELF-QUIZ**

[Self-quiz: Serologic Testing](#)

[Answers: Serologic Testing](#)

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