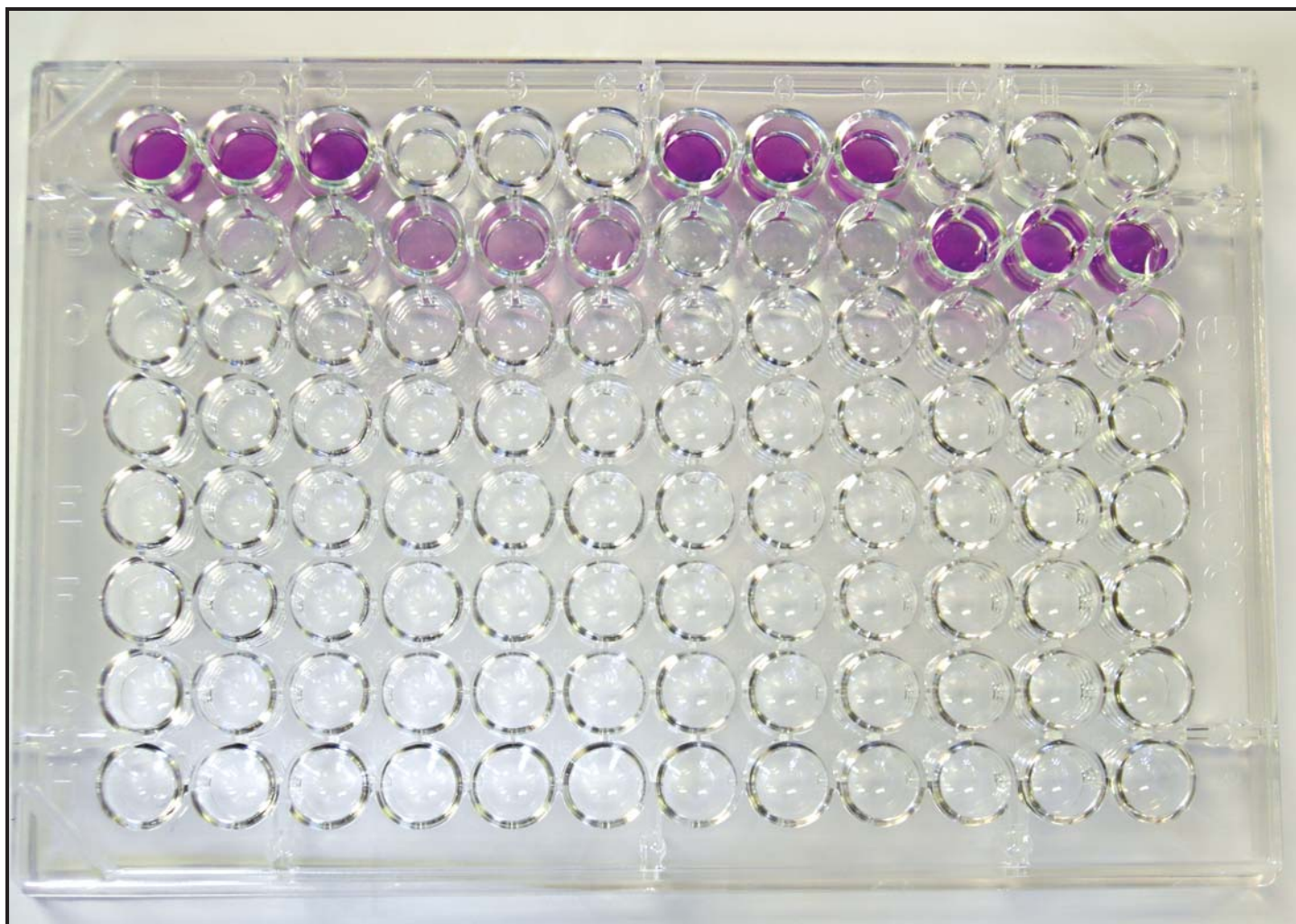


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# ELISA Simulation Kit

TEACHER'S MANUAL WITH STUDENT GUIDE



**CAROLINA**  
World-Class Support for Science & Math

# ELISA Simulation Kit

## Teacher's Manual

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**Note:** Upon receipt, store samples A–F and the positive and negative controls in a freezer (approximately  $-20^{\circ}\text{C}$ ). If a freezer is unavailable, these materials can be stored in a refrigerator (approximately  $4^{\circ}\text{C}$ ) for up to one month. All other materials may be stored at room temperature (approximately  $25^{\circ}\text{C}$ ).

# ELISA Simulation Kit

## Overview

Using the ELISA Simulation Kit, students explore how the principles of antibody-based human immunity apply to a common laboratory test called ELISA (enzyme-linked immunoabsorbant assay). ELISA is commonly used to test blood serum for the presence of antibodies against disease-causing pathogens such as viruses and bacteria. In this way, the assay indirectly detects infection by particular disease-causing agents. Students work in pairs to perform a hands-on simulation of an indirect ELISA using simulated reagents. Students take an investigative approach to diagnose six fictitious patients for either HIV, Lyme Disease, Avian Influenza (Bird Flu), or West Nile virus. Hypothetical scenarios are provided for each patient being tested for each disease. This kit is a simulated ELISA and does not include actual serum samples, antibodies, antigens, or disease agents. As such, there is no risk of infection from the materials included in this kit.

## Objectives

- To understand the basic principles of antibody-mediated immunity
- To understand the steps involved in performing an ELISA and how it is used as a diagnostic tool
- To understand the disease-causing agent and transmission patterns of certain infectious diseases

## Background

### The Body's Defense System

The body possesses several lines of defense against infection by pathogenic organisms. Pathogens are tiny, disease-causing agents including viruses, bacteria, protozoa, molds, and other microorganisms. Pathogens invade the body and multiply; they can cause sickness or even the death of the invaded individual. The body employs three lines of defense to prevent and fight off such dangerous intrusions. The first two defense modes are nonspecific. They include the body's physical barriers and the nonspecific immune system. These defenses function without regard to the type of pathogenic intruder. The third layer of defense is the body's specific immune system. Specific immune responses are tailored to the type of invading pathogen.

### *Nonspecific Barriers*

The primary defense against intruding pathogens is the external protective covering of the body, the skin, and the mucous membranes that line the mouth, nostrils, and other potential gateways. This armor presents a nonspecific physical barrier to foreign invaders. Secretions from the skin and mucous membranes, such as sweat, tears, and saliva, also inhibit entry into the body.

### *Nonspecific Immune Response*

If the body's exterior barriers are breached, several internal, nonspecific immune defenses are launched. One such response is a fever, a rise in body temperature. Many pathogens cannot function well at body temperatures even slightly higher than normal. A fever, therefore, can slow the spread of an infection.

The inflammatory response is another nonspecific immune defense. This response increases blood flow to an infected area, resulting in localized redness and swelling. White blood cells called neutrophils rush to the area of infection as part of the blood flow. They engulf and destroy invading organisms by phagocytosis. Other white blood cells called monocytes develop into macrophages, which are large phagocytic cells that also ingest intruding pathogens.

Another nonspecific internal defense includes antimicrobial proteins of the complement system and antiviral interferon proteins. These proteins act to directly destroy some pathogens and enhance other mechanisms of immunity.

Although the nonspecific immune system indiscriminately attacks invading pathogens, it can distinguish its own cells (self) from foreign cells (non-self). It can also recognize and destroy abnormal body cells—cells that could lead to cancer. Natural killer cells play a role in nonspecific cell-mediated immunity by attacking abnormal body cells and infected cells.

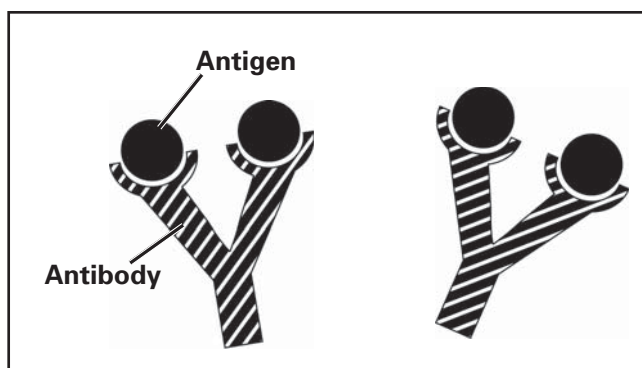
### ***Specific Immune Response***

Invading microbes also encounter the force of the specific immune system. The specific immune system is complex and involves several organs and tissues, including the thymus, spleen, lymph nodes, bone marrow, and white blood cells. This section focuses on the aspects of specific immunity that apply to the ELISA test discussed in the next section.

Specific immune responses are triggered by antigen molecules. Antigens include proteins and other molecules produced by pathogens. The key players in the specific immune defense are dendritic cells, macrophages, and small white blood cells called B lymphocytes (B cells) and T lymphocytes (T cells).

Phagocytic macrophages and dendritic cells break down pathogens and display antigenic fragments from the pathogens on the surface of their cell membranes. B and T lymphocytes circulate through the body in the blood and lymph. When T cells see displayed antigenic fragments, they stimulate specific B cells to reproduce and generate antibodies designed against the specific structure of the antigen encountered. Thus, the word *antigen* is derived from the term “antibody generator.”

Antibodies are a group of serum proteins (also referred to as immunoglobulins) that are found in the bloodstream or bound to cell membranes. These proteins all have the same basic Y-shaped structure, but have different antigen binding sites at their ends. Antigen binding sites are designed to fit the shape of specific antigens. Antibodies bind to antigens like a lock and key, forming antigen-antibody complexes (see Figure 1).



**Figure 1.** Antigen-antibody complex

When an antibody forms an antigen-antibody complex, generally it marks the invading organism/antigen for destruction or for clearance from the bloodstream by phagocytic cells. This removal is designed to prevent the organism/antigen from infecting the cell. Antigen-antibody complexes also stimulate additional immune responses to aid the body in clearing an infection.

The specific immune system has an incredible ability to learn and remember. The first time the body is exposed to an antigen, a primary immune response is launched and antibody-producing B cells and T cells work steadily over time to eliminate the infection. If the same antigen is encountered a second time, the body is primed and remembers how to respond. It launches a more potent secondary immune response that can rid the body of the invading antigen more quickly. This is known as immunological memory.

## ELISA

Scientists have applied the basic principles of antibody-mediated immunity to an assay for detecting infection by specific organisms. This assay is called an ELISA (enzyme-linked immunoabsorbant assay) and is based on the principle that antibodies produced in response to pathogens attach to their antigen targets with great specificity to form antigen-antibody complexes. ELISAs can be used to test for infection by HIV, influenza virus, the bacterium that causes Lyme Disease, smallpox virus, SARS coronavirus, West Nile virus, and other disease agents.

There are two types of ELISA tests—direct ELISA and indirect ELISA. Indirect ELISA is used to detect infection by testing patients' blood for the presence or absence of antibodies against a particular pathogen. The presence of such antibodies indicates that the individual has been infected and that their body has launched an immune response against the disease-causing agent. Direct ELISA assays for the presence or absence of certain antigens in patients' blood. The lab performed in this kit simulates the indirect ELISA test.

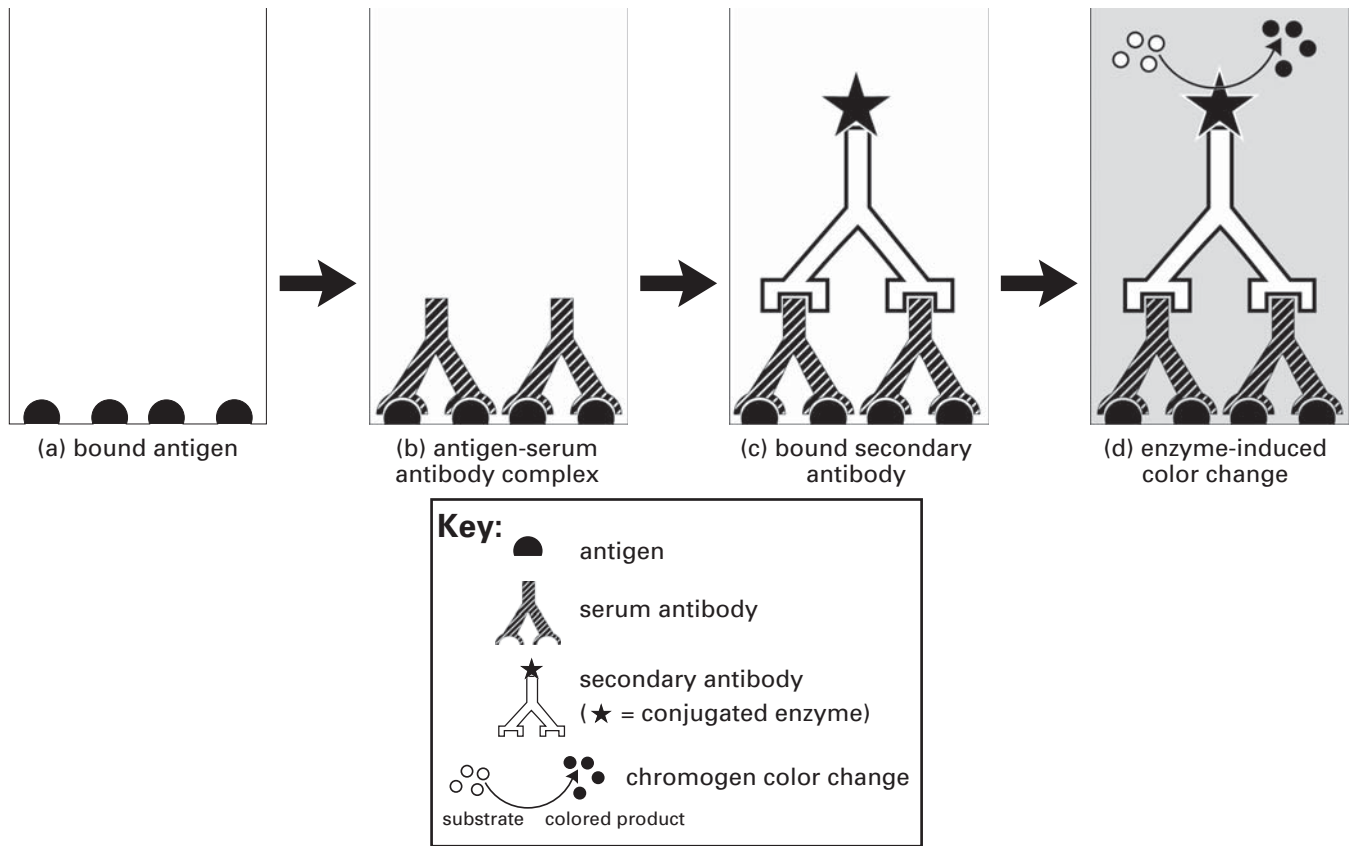
In the first step of an indirect ELISA, antigen proteins purified from the infectious agent, or genetically engineered versions of the antigens, are added to the wells of plastic microtiter plates. These antigen proteins bind to the bottom of the well by forming hydrophobic associations with the plastic surface (see Figure 2a). The wells of the plate are then washed with a buffer to remove any unbound material.

Next, blood serum from the patient(s) being tested is added to the treated wells. If these serum samples contain antibodies against the bound antigen, the antibodies will attach to the antigens, forming tight complexes (see Figure 2b). Such antigen-antibody complexes are not visible by eye, so detection steps (described in the next paragraph) must be employed to visualize them. The wells are again washed to remove any unbound proteins.

Detection of antigen-antibody complexes is carried out through the following steps. A secondary antibody that recognizes antibodies produced by humans (anti-human antibody) is added to the wells. If antigen-antibody complexes formed in the wells, this secondary antibody recognizes and binds to the primary antibodies from the patients' serum (see Figure 2c). The secondary antibody is attached to an enzyme that will facilitate the final detection. (This antibody/enzyme combination is called a conjugate.) The wells are rinsed one last time to remove unbound molecules.



In the final step, a chromogen substrate is added to the wells of the plate. If present, the enzyme that is linked to the secondary antibody facilitates a chemical reaction that changes the color of the chromogen (see Figure 2d). A color change indicates that the patient possesses antibodies to the antigen and has been infected. No change in color indicates that the patient has not been infected, or that their body has not yet launched an immune response to produce antibodies against the invading antigen.



**Figure 2.** ELISA schematic of a positive result

In this simulated assay, each sample will be tested in triplicate to ensure reproducibility. Known positive and negative samples are included as controls. For ease of performance, the well washing steps of a true ELISA have been eliminated in this simulation. It is important to note that the washes are a necessary step of an actual assay. Likewise, it is critical to use a clean pipet for each new sample or reagent to prevent cross-contamination of the wells.

## Materials

**Note:** This kit contains a coupon for prepaid delivery of perishable materials (noted below with an asterisk). Refer to the materials request form for more information.

**Note:** Upon receipt, store samples A–F and the positive and negative controls in a freezer (approximately  $-20^{\circ}\text{C}$ ). If a freezer is unavailable, these materials can be stored in a refrigerator (approximately  $4^{\circ}\text{C}$ ) for up to one month. All other materials may be stored at room temperature (approximately  $25^{\circ}\text{C}$ ).

The materials in this kit are designed for a class of 32 students working in pairs. The materials provided are designed for use with the ELISA Simulation Kit only. Carolina Biological Supply Company disclaims all responsibility for any other use of these materials.

### *Included in the kit*

positive control, 6 mL\*

negative control, 6 mL\*

Patient A sample, 6 mL\*

Patient B sample, 6 mL\*

Patient C sample, 6 mL\*

Patient D sample, 6 mL\*

Patient E sample, 6 mL\*

Patient F sample, 6 mL\*

simulated antigen, 30 mL

simulated secondary antibody, 30 mL

simulated chromogen, 30 mL

16 microtiter plates

180 plastic pipets

## Teacher Tips

- This kit is designed to simulate the steps and visual results of an ELISA. It does not include actual serum samples, antibodies, or antigens. As such, there are no blood products or disease-causing agents, and there is no risk of infection from the materials included in this kit.
- This kit contains four different patient profile sheets, each describing a different disease. Each student pair requires one patient profile sheet of your choosing. You can distribute the same profile sheet to the entire class, or give different sheets to different pairs of students. This design provides variety and versatility in exploring the diagnosis of infectious diseases by ELISA.
- In this simulated ELISA, all reaction wells will turn light green when the chromogen is added. A change in color from light green to purple indicates a positive result.
- The colorimetric results of the assay should be analyzed after incubation for a minimum of 5 minutes and a maximum of 10 minutes. Shorter incubation will result in incomplete development of the weak positive result. Longer incubation will result in continued development and deepening color, which may make it difficult for students to distinguish a strong and a weak positive result.
- When reading the ELISA results, you may find it helpful to place the microtiter plate on a sheet of white paper to provide contrast.

## Teacher Preparation

- If the samples have been frozen, allow them to thaw at room temperature before use. Thawing should take approximately 30 minutes. Samples can be kept at room temperature throughout the lab.
- The reagents included in this kit must be shared among the entire class. If you wish to aliquot these reagents, each pair of students needs the following quantities:  
 300  $\mu\text{L}$  each of the positive control, negative control, and patient A–F samples  
 1500  $\mu\text{L}$  each of simulated antigen, simulated secondary antibody, and simulated chromogen
- Distribute 1 microtiter plate and 11 plastic pipets to each pair of students.
- Photocopy the appropriate profile sheet(s) for the disease(s) you wish to discuss. Distribute one profile sheet to each student pair.
- Photocopy the Student Guide at the end of this manual and distribute a copy to each student or pair of students.

## Instructions

Refer to the Student Guide for step-by-step activity instructions.

## Expected Results

The cover of this Teacher's Manual shows a color photograph of the expected results of this laboratory exercise.

### Data Table Answer Key

Sample	Color	Test Result
positive control	dark purple	positive
negative control	light green	negative
Patient A	<i>dark purple</i>	<i>positive</i>
Patient B	<i>light green</i>	<i>negative</i>
Patient C	<i>light green</i>	<i>negative</i>
Patient D	<i>light purple</i>	<i>weak positive</i>
Patient E	<i>light green</i>	<i>negative</i>
Patient F	<i>dark purple</i>	<i>positive</i>

## Answers to Questions in the Student Guide

1. What basic principles of antibody-mediated immunity are utilized in an ELISA assay?  
*ELISA assays are based on the principles that antibodies are produced in response to infection and that these antibodies are designed to specifically target particular antigens and bind tightly to them.*
2. How does an ELISA indirectly detect infection by a disease-causing agent?  
*ELISA tests indirectly detect infection by assaying patients' blood serum for antibody molecules designed to target the infectious agent in question.*



3. What is the function of the secondary antibody and chromogen in an ELISA?  
*Antigen-antibody complexes formed in the initial steps of an ELISA are not visible to the unaided eye. Therefore, a colorimetric detection system involving a secondary antibody and chromogen is employed. The secondary antibody, which is conjugated to an enzyme, recognizes and binds to primary antibodies of antigen-antibody complexes, if they are present. Chromogen substrate is then added. If present, the enzyme linked to the secondary antibody changes the color of the chromogen. A color change indicates that the patient has antibodies against the pathogen and has been infected.*
4. Why did you perform three identical tests for each control and patient sample?  
*Each assay was performed in triplicate to ensure reproducibility of the results.*
5. What might cause some positive results to be lighter in color than others?  
*Weak positive results may be an indication that the patient's blood serum carries few antibodies against the disease-causing agent. The patient's exposure to the pathogen may be recent and the body may not have launched a full immune response yet. Alternatively, the infection may have occurred long ago, and the level of antibodies in the patient's bloodstream is declining. (Note: Other explanations are possible and acceptable, but these are most likely.)*
6. Describe the disease-causing pathogen for the disease you assayed for, including its mode of transmission to people.  
*Answers will vary. The disease-causing agent for HIV, West Nile virus, and Influenza are different viruses. The disease-causing pathogen for Lyme Disease is the bacterium, Borrelia burgdorferi. HIV can be transmitted from person to person through exchange of body fluids. Lyme Disease can be transmitted to people through the bite of an infected tick. West Nile virus can be transmitted to people through the bite of an infected mosquito. Although there have been few cases as of early 2006, Avian Influenza can be transmitted to people through close contact with infected domestic poultry.*
7. Write your reaction to the diagnosis of the patients you tested. Were you surprised by the outcome of the test based on the scenarios provided? How do you think infection could have been prevented in the patients who tested positive?  
*Answers will vary.*

## Patient Profiles for HIV Testing

HIV stands for Human Immunodeficiency Virus. It is the virus responsible for causing the Acquired Immune Deficiency Syndrome, or AIDS. HIV attacks and destroys a type of T lymphocyte of the immune system called CD4<sup>+</sup> cells, and thereby severely weakens immune defense. Shortly after infection by HIV, the body begins producing antibodies against the virus, and the infected person may feel flu-like symptoms. The virus then enters a latent phase where the infected person does not experience symptoms but the virus and the immune cells continue to attack each other. Eventually, the HIV virus gains the upper hand. The immune system loses its ability to fight off the opportunistic infections it could normally combat, and advanced AIDS results. HIV is communicable and can be transmitted from person to person through blood, pregnancy, breast milk, and other bodily fluids.

### Instructions

Read the following patient profiles. Perform an ELISA (simulation) to test each individual for antibodies against HIV.

#### Patient A

Patient A has just learned that her ex-boyfriend was diagnosed with HIV. Although they often used protection when sexually active, she is concerned that she could have contracted the virus from him.

#### Patient B

Patient B is the current boyfriend of Patient A. Although they are not sexually active, they have engaged in deep kissing. Patient B is concerned for his girlfriend and for himself, and decides to get tested along with her.

#### Patient C

Patient C is an EMT who treated a bleeding car accident victim who was HIV positive. After attending to the accident victim's injuries, he realized that his glove was torn and his hand cut.

#### Patient D

Patient D just broke up with her boyfriend after learning about several lies he told her. She worries that he has not been monogamous and decides to be tested for HIV.

#### Patient E

Patient E is happily married with young children when he gets a call from an old college girlfriend who is HIV positive. She does not know when she contracted the virus and is contacting all her old partners so that they can be tested as well.

#### Patient F

Patient F experimented with drugs during college and used some intravenous substances. Although he has been clean and sober for years, he remembers sharing a needle with a friend at a social function and decides to be tested for HIV.

## Patient Profiles for Lyme Disease Testing

The pathogenic agent of Lyme Disease is a spirochete bacterium, *Borrelia burgdorferi*. The bacterium is transmitted to people through the bite of infected ticks, such as deer ticks and black-legged ticks. It cannot be transferred from person to person. The life cycle stages of ticks require meals of blood obtained from warm-blooded animals. When an infected tick bites a victim, it transmits the infectious bacteria after 36–48 hours of attachment. An immune response is then launched in the infected individual. Early removal of ticks can prevent such transmission of infection. Early symptoms of Lyme Disease include a characteristic bull's eye rash (erythema migrans) surrounding the bite, and flu-like symptoms such as fever, headache, chills, muscle aches, and fatigue. If the disease goes untreated, it can result in more serious symptoms such as severe muscle pain, extreme fatigue, neurological difficulties, and respiratory and gastrointestinal problems.

### Instructions

Read the following patient profiles. Perform an ELISA (simulation) to test each individual for antibodies against *Borrelia burgdorferi*.

#### Patient A

Patient A went on a hike with her hiking club and has since felt feverish and achy. She is an avid hiker who knows the risks of Lyme Disease and always uses insect spray and wears protective clothing. She thinks she probably has the flu.

#### Patient B

Patient B was gardening in her yard when she noticed a tick on her arm. She carefully removed the tick but has been feeling tired since. Although she does not have a bull's eye rash, she gets tested for Lyme Disease.

#### Patient C

Patient C has just learned that her best friend from summer camp has Lyme Disease. Although the disease cannot be transferred from person to person, she could have been exposed to other infected ticks in the area.

#### Patient D

Patient D went on a winter hike on a warm day after a snowfall and has been feeling ill since. The patient feels certain that he has the flu, but his doctor notices a bull's eye rash on his neck.

#### Patient E

Patient E cleared a large quantity of brush at his home and noticed a solid, red rash on his leg days after. He has also been suffering from headaches and fatigue.

#### Patient F

Patient F has been feeling ill for quite some time and his doctors cannot figure out why. He is extremely tired and complains of terrible muscle and joint pain. He has not been outdoors much recently and has no rashes on his body.

## Patient Profiles for Avian Influenza Testing

Throughout history, influenza (commonly called “the flu”) has plagued society and caused pandemic outbreaks of disease. The flu is caused by the influenza virus. There are many different strains of the influenza virus, which cause different variations of influenza disease. Recombination of influenza virus’s genetic material enables the virus to take on many forms, which poses a significant challenge to vaccination. The seasonal flu that we are familiar with is caused by different human influenza virus strains, such as H3N2, H1N1, and H1N2.

There are also strains of the influenza virus that affect animals, pigs and birds, for example. When influenza viral strains in animals mutate in a way that enables them to infect people, they are particularly virulent because the human immune system has little protection against genetically new viruses derived from animal hosts. The greatest cause for concern is when these lethal viral strains further change to become transmissible from person to person. Such influenza strains become highly contagious and can spread quickly throughout a population.

Avian Influenza (Bird Flu) caused by the H5N1 strain of the influenza virus is highly contagious among birds and can cause domestic poultry such as chickens, ducks, and turkeys to become very sick and die shortly thereafter. News of H5N1 affecting domestic poultry was first reported in Europe and Asia. As of mid-2006, there have been relatively few cases of the virus crossing the species barrier to infect humans who have had contact with infected birds. Thus far, person-to-person transmission has been limited and has not continued beyond one individual.

### Instructions

Read the following patient profiles. Perform an ELISA (simulation) to test each individual for antibodies against the H5N1 avian influenza virus.

#### Patient A

A poultry vendor in Asia has noticed that many of his chickens have been exhibiting ruffled feathers and a decrease in egg production. Patient A is one of the vendor’s sickly chickens.

#### Patient B

Patient B is an employee of the poultry vendor. His job is to care for and tend to a large number of chickens, including Patient A.

#### Patient C

Patient C is the wife of Patient B. She does not have any contact with the chickens but she regularly washes her husband’s work clothes and lives in a small house with him.

#### Patient D

Patient D is a young boy who feeds and cares for the chickens at his home in central Europe. He has suddenly become quite ill. Several of the chickens in his flock are also sick.

#### Patient E and Patient F

Patient E and Patient F are two chickens from the flock that Patient D tends.

## Patient Profiles for West Nile Virus Testing

West Nile virus mainly infects corvids (birds such as jays, crows, and magpies) and robins. Although birds are the primary and preferred host of the virus, it can be transferred to humans when a mosquito bites an infected bird and then bites a person. An infected mosquito injects the West Nile virus from its salivary glands when it bites. This is the primary route of human infection, although the virus can be transferred from person to person through organ transplants, blood transfusions, prenatal infections, and through breast milk. Most infected persons (80%) do not experience symptoms, but a smaller portion (20%) exhibit West Nile fever with mild flu-like symptoms. In rare cases, the virus crosses the blood-brain barrier causing more serious problems such as encephalitis (brain inflammation), meningitis (inflammation of the lining of the brain and spinal cord), and temporary blindness. No effective treatment for the disease is known. West Nile virus is especially fatal to crows and other corvids. A large proportion of dead crows in an area can be an indication of West Nile virus prevalence.

### Instructions

Read the following patient profiles. Perform an ELISA (simulation) to test each individual for antibodies against West Nile virus.

#### Patient A

Patient A lives in a community where a bird infected with West Nile virus was discovered. She went on a camping trip recently and was bitten by several mosquitoes. She feels feverish and has some flu-like symptoms. Her doctors want to test her for West Nile virus.

#### Patient B

Patient B is the 8-month-old son of Patient A. Patient A breast-feeds her son. Her doctors tell her that if she is infected, she could pass the virus to her son while he is nursing. Rather than wait for the results of her own test, she decides to have her son tested for West Nile virus immediately.

#### Patient C

Patient C was on the same camping trip as Patient A. He has been feeling fine but is covered in mosquito bites. He is worried that one of his bites could have been from an infected mosquito.

#### Patient D, Patient E, and Patient F

A farmer has noticed a large number of dead crows in his fields. His son has been experimenting with different ways to keep crows away from their crops, and some of these methods have proven fatal to the birds. Nevertheless, the farmer has heard reports of local birds carrying West Nile virus, and he is concerned. Blood samples from three dead birds found on his property are tested for West Nile virus. The three birds are referred to as Patient D, Patient E, and Patient F.

# ELISA Simulation

## Introduction

### The Body's Defense System

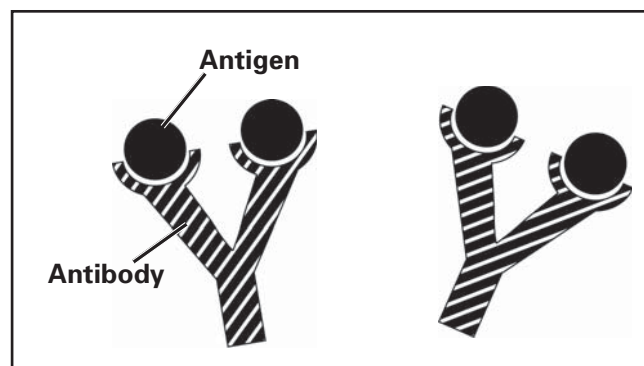
The body possesses several lines of defense against infection by pathogenic organisms. Pathogens are tiny, disease-causing agents including viruses, bacteria, protozoa, molds, and other microorganisms. Pathogens invade the body and multiply; they can cause sickness or even the death of the invaded individual. The body employs three lines of defense to prevent and fight off such dangerous intrusions. The first two defense modes are nonspecific. They include the body's physical barriers and the nonspecific immune system. These defenses function without regard to the type of pathogenic intruder. The third layer of defense is the body's specific immune system. Specific immune responses are tailored to the type of invading pathogen.

### Specific Immune Response

Specific immune responses are triggered by antigen molecules. Antigens include proteins and other molecules produced by pathogens. The key players in the specific immune defense are dendritic cells, macrophages, and small white blood cells called B lymphocytes (B cells) and T lymphocytes (T cells).

Phagocytic macrophages and dendritic cells break down pathogens and display antigenic fragments from the pathogens on the surface of their cell membranes. B and T lymphocytes circulate through the body in the blood and lymph. When T cells see displayed antigenic fragments, they stimulate specific B cells to reproduce and generate antibodies designed against the specific structure of the antigen encountered. Thus, the word *antigen* is derived from the term "antibody generator."

Antibodies are a group of serum proteins (also referred to as immunoglobulins) that are found in the bloodstream or bound to cell membranes. These proteins all have the same basic Y-shaped structure, but have different antigen binding sites at their ends. Antigen binding sites are designed to fit the shape of specific antigens. Antibodies bind to antigens like a lock and key, forming antigen-antibody complexes (see Figure 1).



**Figure 1.** Antigen-antibody complex

When an antibody forms an antigen-antibody complex, generally it marks the invading organism/antigen for destruction or for clearance from the bloodstream by phagocytic cells. This removal is designed to prevent the organism/antigen from infecting the cell. Antigen-antibody complexes also stimulate additional immune responses to aid the body in clearing an infection.



## ELISA

Scientists have applied the basic principles of antibody-mediated immunity to an assay for detecting infection by specific organisms. This assay is called an ELISA (enzyme-linked immunoabsorbant assay) and is based on the principle that antibodies produced in response to pathogens attach to their antigen targets with great specificity to form antigen-antibody complexes.

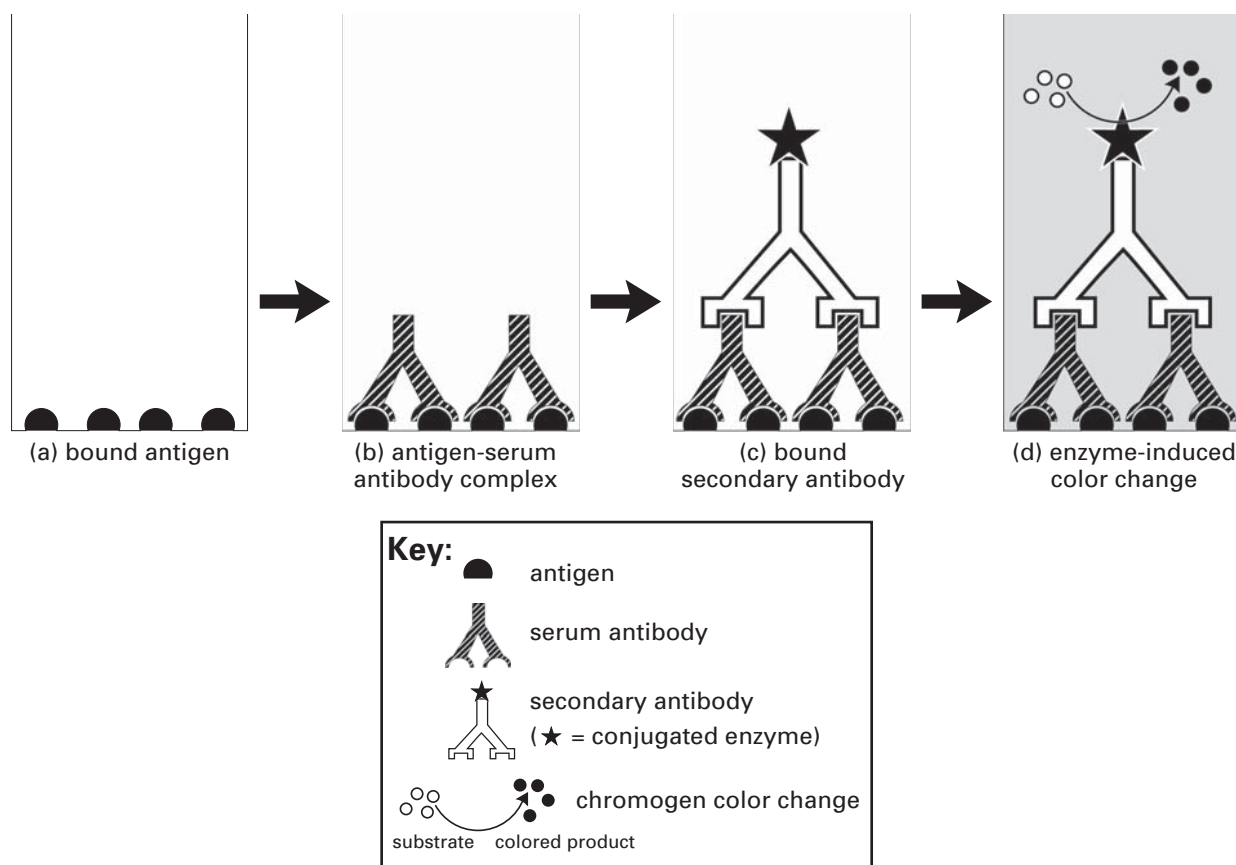
There are two types of ELISA tests—direct ELISA and indirect ELISA. Indirect ELISA is used to detect infection by testing patients' blood for the presence or absence of antibodies against a particular pathogen. The presence of such antibodies indicates that the individual has been infected and that their body has launched an immune response against the disease-causing agent.

In the first step of an indirect ELISA, antigen proteins purified from the infectious agent, or genetically engineered versions of the antigens, are added to the wells of plastic microtiter plates. These antigen proteins bind to the bottom of the well by forming hydrophobic associations with the plastic surface (see Figure 2a). The wells of the plate are then washed with a buffer to remove any unbound material.

Next, blood serum from the patient(s) being tested is added to the treated wells. If these serum samples contain antibodies against the bound antigen, the antibodies will attach to the antigens, forming tight complexes (see Figure 2b). Such antigen-antibody complexes are not visible by eye, so detection steps (described in the next paragraph) must be employed to visualize them. The wells are again washed to remove any unbound proteins.

Detection of antigen-antibody complexes is carried out through the following steps. A secondary antibody that recognizes antibodies produced by humans (anti-human antibody) is added to the wells. If antigen-antibody complexes formed in the wells, this secondary antibody recognizes and binds to the primary antibodies from the patients' serum (see Figure 2c). The secondary antibody is attached to an enzyme that will facilitate the final detection. (This antibody/enzyme combination is called a conjugate.) The wells are rinsed one last time to remove unbound molecules.

In the final step, a chromogen substrate is added to the wells of the plate. If present, the enzyme that is linked to the secondary antibody facilitates a chemical reaction that changes the color of the chromogen (see Figure 2d). A color change indicates that the patient possesses antibodies to the antigen and has been infected. No change in color indicates that the patient has not been infected, or that their body has not yet launched an immune response to produce antibodies against the invading antigen. Positive results determined by ELISA undergo a different test to confirm the findings.



**Figure 2.** ELISA schematic of a positive result

In this simulated assay, each sample will be tested in triplicate to ensure reproducibility. Known positive and negative samples are included as controls. For ease of performance, the well washing steps of a true ELISA have been eliminated in this simulation. It is important to note that the washes are a necessary step of an actual assay. Likewise, it is critical to use a clean pipet for each new sample or reagent to prevent cross-contamination of the wells.

## Instructions

1. Using one plastic pipet, carefully administer 3 drops of simulated antigen in each well of rows A and B of the microtiter plate. Discard the pipet after use. In a true ELISA assay, the antigen would bind to the bottom of the microtiter plate. The wells would then be washed with a buffer to remove any unbound molecules. In this simulated lab activity, the washing step has been eliminated.
2. Using a clean pipet, add 3 drops of positive control to wells A1, A2, and A3 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
3. Using a clean pipet, add 3 drops of negative control to wells A4, A5, and A6 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
4. Using a clean pipet, add 3 drops of Patient A sample to wells A7, A8, and A9 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

5. Using a clean pipet, add 3 drops of Patient B sample to wells A10, A11, and A12 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
6. Using a clean pipet, add 3 drops of Patient C sample to wells B1, B2, and B3 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
7. Using a clean pipet, add 3 drops of Patient D sample to wells B4, B5, and B6 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
8. Using a clean pipet, add 3 drops of Patient E sample to wells B7, B8, and B9 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
9. Using a clean pipet, add 3 drops of Patient F sample to wells B10, B11, and B12 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use. In a true ELISA assay, at this point, the wells would be washed with a buffer to remove any molecules that have not bound to the adhered antigen. In this simulated lab activity, the washing step has been eliminated.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+C	+C	+C	-C	-C	-C	A	A	A	B	B	B
B	C	C	C	D	D	D	E	E	E	F	F	F

+C = positive control, -C = negative control, A-F = Patients A-F

**Figure 3.** Microtiter plate sample wells

10. Using a clean pipet, add 3 drops of simulated secondary antibody to each well of rows A and B on the microtiter plate. Keep track of each well as the reagent is added and do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use. In a true ELISA, the wells would again be washed to remove unbound molecules. This step has been eliminated from this simulation.
11. Using the remaining clean pipet, add 3 drops of simulated chromogen to each well of rows A and B on the microtiter plate. Keep track of each well as the reagent is added and do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.
12. All the reaction wells will turn light green when the chromogen is added. A change from light green to purple indicates a positive result. Incubate the microtiter plate at room temperature for a minimum of 5 minutes and a maximum of 10 minutes to allow for color development.
13. After 5–10 minutes of incubation, record your results in the data table on page S-5. Compare the color of each patient sample to that of the positive and negative control. Colored results that are in between the positive and negative control should be scored as weak positives.

## ELISA Simulation

### Data Table

Sample	Color	Test Result
positive control	dark purple	positive
negative control	light green	negative
Patient A		
Patient B		
Patient C		
Patient D		
Patient E		
Patient F		

### Questions

1. What basic principles of antibody-mediated immunity are utilized in an ELISA assay?
2. How does an ELISA indirectly detect infection by a disease-causing agent?
3. What is the function of the secondary antibody and chromogen in an ELISA?

4. Why did you perform three identical tests for each control and patient sample?
  
  
  
  
  
  
  
  
  
  
5. What might cause some positive results to be lighter in color than others?
  
  
  
  
  
  
  
  
  
  
6. Describe the disease-causing pathogen for the disease you assayed for, including its mode of transmission to people.
  
  
  
  
  
  
  
  
  
  
7. Write your reaction to the diagnosis of the patients you tested. Were you surprised by the outcome of the test based on the scenarios provided? How do you think infection could have been prevented in the patients who tested positive?

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