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Edvo-Kit #

S-70

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How Does a Doctor Test for AIDS?

Experiment Objective:

The Human Immunodeficiency Virus (HIV) is an infectious agent that causes Acquired Immunodeficiency Syndrome (AIDS) in humans. In this simulation, students will perform an ELISA assay, a common test used by doctors to diagnose an HIV infection.

See page 3 for storage instructions.

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Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/Safety-Data-Sheets**

Experiment Components

Contents		Check (✓)
A	HIV Antigens	❑
B	Negative Control	❑
C	Positive Control	❑
D	Donor 1 Serum	❑
E	Donor 2 Serum	❑
F	Secondary Antibody (2°Ab)	❑
G	Substrate	❑
•	HIV Antibody Detection Strips	❑
•	Small Transfer Pipets	❑
•	Large Transfer Pipets	❑
•	Microcentrifuge Tubes	❑

Experiment #S-70 is designed for 10 groups.

Storage:
Store experiment at room temperature

Requirements

- Distilled Water
- Beakers (50 or 100 ml)
- Pencils
- Gloves
- Timer or Clock
- Paper Towels

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

This experiment does not contain HIV virus or its components. None of the components have been prepared from human sources

Background Information

HIV DETECTION BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The Human Immunodeficiency Virus (HIV) is an infectious agent that causes Acquired Immunodeficiency Syndrome (AIDS) in humans (Figure 1). Most infectious human viruses have DNA as the genetic material, but the HIV-1 virus has its genetic information encoded in RNA. HIV infects both males and females of all ages. Since HIV is found in blood and other body fluids, infection is possible through direct exposure to these fluids. Blood banks in the United States check for the HIV-1 virus to avoid such infections.

The immune system is the body's primary defense against infections by bacteria and viruses. If the human immune system is compromised, it does not have ability to fight off opportunistic infections, such as bacterial skin infections, viral induced skin cancers, and respiratory infections such as pneumonia.

There are several diagnostic procedures to determine if a person is infected by HIV-1. Although there is currently no cure for AIDS, early detection can result in treatment with drugs which inhibit the virus from spreading the infection further, and can extend the life of a patient. The Enzyme linked Immunosorbent assay (ELISA) test is used as the initial screening test to detect blood samples containing HIV antigens.

In an ELISA test, a series of steps are performed to determine if an individual has mounted an antibody response to the HIV virus (antigen). This is determined by a test in which a colorless sample turns to a color if the test is positive. If the antibody against the virus is not present, then there will be no color change (negative). Another test, called Western blot analysis, is performed as a secondary test to confirm HIV infection if positive ELISA test results are obtained.

The steps performed in an ELISA are usually performed in plastic microtiter plates. These transparent plates contain many small wells in which the liquid samples are pipeted. First, the antigen is added to the wells and incubated. After washing away the excess antigen, some antigen is adsorbed to the walls of the wells. Next a blocking substance such as milk protein is added to block sites not occupied by the antigen. Then serum containing IgG antibodies is added. If the antibodies are specific for the antigen or positive then they will bind to the adsorbed antigens in the wells. Next, a secondary antibody (Anti-IgG) is added to the well where it binds to the primary antibody. The secondary antibody is cross-linked to the enzyme horseradish peroxidase. The antigen-antbody complex is detected by adding a colorless substrate solution to each well. A color change is observed in any samples positive for HIV antibodies.

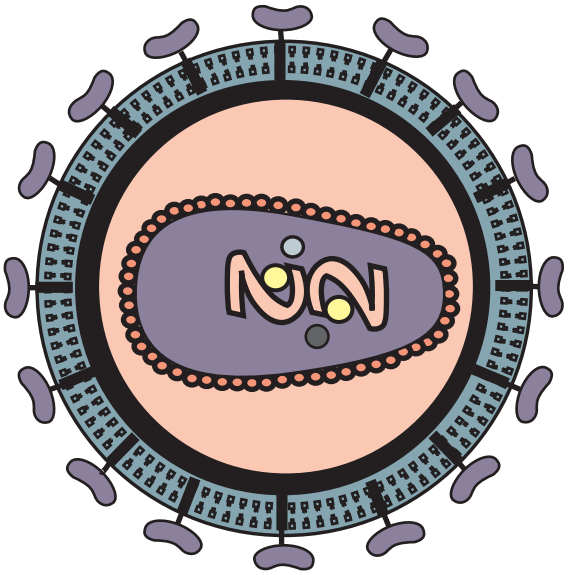


Figure 1: Overview of HIV Structure

Background Information

This ELISA simulates the steps a clinician would take to detect an HIV infection by looking for HIV antibodies in the patient's blood (Figure 2). To perform the ELISA, the microtiter plate is coated with HIV antigens. Actual ELISA tests are generally done in clear plastic microtiter plates. Each plate contains many small wells, in which the samples are deposited. This experiment uses an HIV Antibody Strip with circles to simulate the microtiter plate. Next, the patient samples are added to the microtiter plate. If HIV antibodies are present in the blood, they will bind to the antigens on the plate. The HIV antibodies are detected with an enzyme-linked secondary antibody that can change the color of a clear substrate. When the substrate is added to the wells, a color change is observed in any samples positive for HIV antibodies

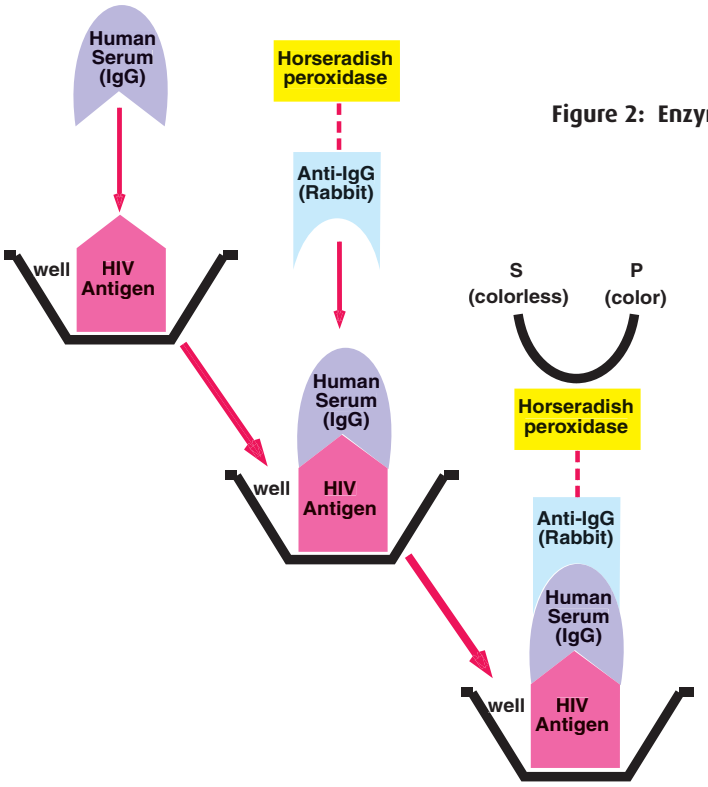


Figure 2: Enzyme-Linked Immunosorbent Assay

Experiment Overview

EXPERIMENT OBJECTIVE:

The Human Immunodeficiency Virus (HIV) is an infectious agent that causes Acquired Immunodeficiency Syndrome (AIDS) in humans. In this simulation, students will perform an ELISA assay, a common test used by doctors to diagnose an HIV infection.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

WORKING HYPOTHESIS

If an individual has been exposed to the HIV virus and mounts an immune response, then a positive result (color change) will be observed when an ELISA test is performed

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Conducting an ELISA Simulation

1. Using a pencil, label the HIV Antibody Test Strip with your group number.

2. Place your test strip on top of a paper towel.

3. Rinse a transfer pipet in a beaker of distilled water. Practice squeezing the pipet slowly to get one drop at a time.

   When you are comfortable with using the pipet, remove any remaining water before starting the experiment.

4. Carefully place one drop of antigen onto each circle of the HIV Antibody Detection Strip.

   Try to get the drop inside the circle, but do not be concerned if it spreads.

   - Replace any unused sample back into the tube.
   - Flush and rinse the pipet thoroughly in distilled water.
   - Incubate the strip for 5 minutes at room temperature.

5. Place one drop of the negative sample ("Neg") onto each of the two negative circles (Row 1).

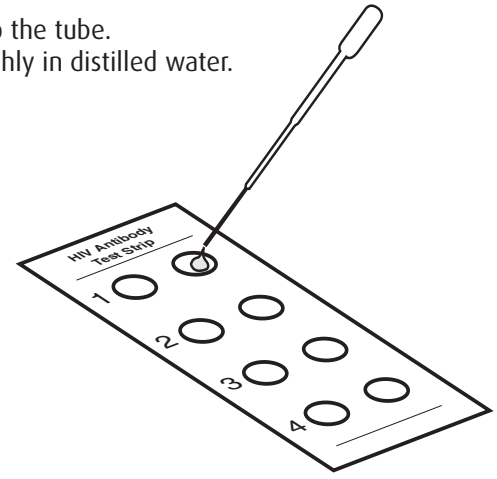
   - Replace unused sample back into the tube.
   - Flush and rinse the pipet thoroughly in distilled water.

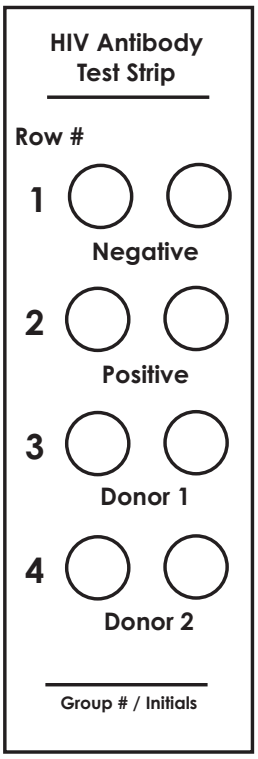
6. Place one drop of the positive sample ("Pos") onto each of the two positive circles (Row 2).

   - Replace unused sample back into the tube.
   - Flush and rinse the pipet thoroughly in distilled water.

7. Place one drop of the "D1" sample onto each of the two Donor 1 circles (Row 3).

   - Replace unused sample back into the tube.
   - Flush and rinse the pipet thoroughly in distilled water.

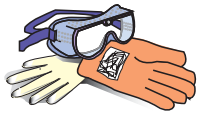




The circles on the HIV Antibody Test strip represent the wells of a plastic microtiter plate.

Conducting an ELISA Simulation

8. Place one drop of the "D2" sample onto each of the two Donor 2 circles (Row 4).
    - Replace unused sample back into the tube.
9. Discard the pipet and incubate the strip for 5 minutes at room temperature.
10. Using a new pipet, place one drop of the 2°Ab (secondary antibody) onto each circle on the strip.
    - Replace unused sample back into the tube.
    - Flush and rinse the pipet thoroughly in distilled water.
    - Incubate the strip for 5 minutes at room temperature.
11. Place one drop of substrate onto each circle on the strip.
12. Observe and record the results. Positive results should appear bright pink.



Wear gloves and safety goggles

Study Questions

1. What is the function of the immune system in the human body?
2. What are T cells?
3. What are antibodies?
4. Why is it necessary to do the experiment in duplicate?
5. What are negative and positive controls?
6. Why is it important to have controls in an experiment?
7. Which control will show that a donor has been exposed to HIV?
8. Based on the ELISA Detection test you conducted, which donor(s) have been exposed to HIV?
9. What are two ways to people can be exposed to HIV and how can they protect themselves?

Instructor's Guide

PRE-LAB PREPARATIONS

Samples may be dispensed the day before the experiment:

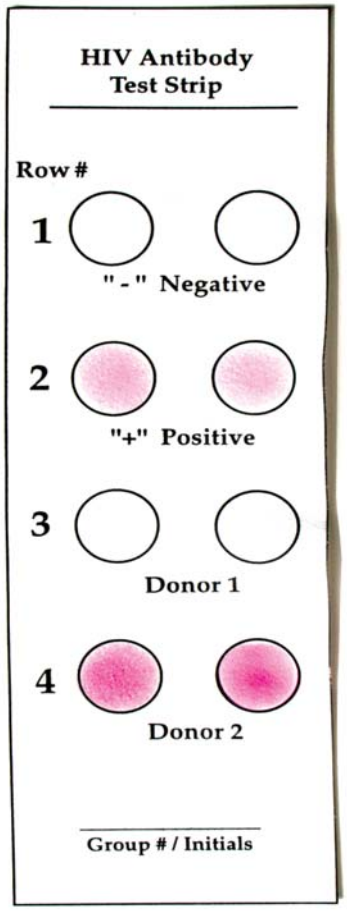
1. Label 10 tubes each for the following:

Component	Label
HIV Antigens	Antigen
Negative control	Neg
Positive control	Pos
Donor 1 Serum	D1
Donor 2 Serum	D2
Secondary Antibody	2°Ab
Substrate	Substrate

2. Use a separate large transfer pipet for dispensing each component to the appropriately labeled tubes.
   - Fill each tube to the 0.5 ml mark on the side of the tube.
   - Cap the tubes and store at room temperature.

3. Cut the HIV Antibody Detection Strips along the solid lines.

Experiment Results



Sample	Result
Negative Control	No color change, HIV negative
Positive Control	Pink color change. HIV positive
Donor 1 Serum	No color change, HIV negative
Donor 2 Serum	Pink color change, HIV positive

Please refer to the kit insert for the Answers to Study Questions