EDVOTEK®

Sci-On® Biology

S-70

EDVO-Kit #

How Does a Doctor Test for AIDS?

Storage:
Store this experiment in the refrigerator.

EXPERIMENT OBJECTIVES:

The objective of this experiment is to learn about basic concepts related to HIV infection, AIDS and the ELISA test, which is the initial screening test for the presence of the HIV virus.

All 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.

Table of Contents

Experiment Components

This experiment is designed for 20 students working in pairs.

Storage:
Store entire experiment in the refrigerator.

Contents

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
Microtest Tubes

None of the experiment components have been prepared from human sources.

Requirements

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

All 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.

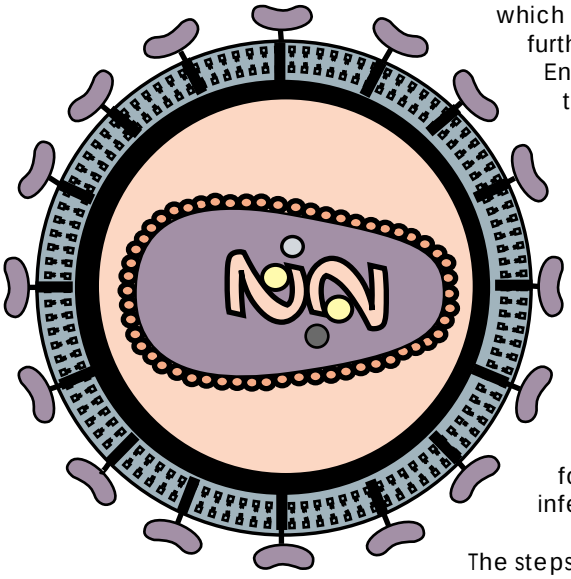
AIDS Detection by Enzyme-Linked Immunosorbent Assay (ELISA)

Background Information

AIDS is a disease in humans initiated by the infection of a virus known as HIV-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. Body fluids such as blood, are a source of infection, either upon direct exposure or through transfusions. Blood banks in the United States check for the HIV-1 virus to avoid such infections.

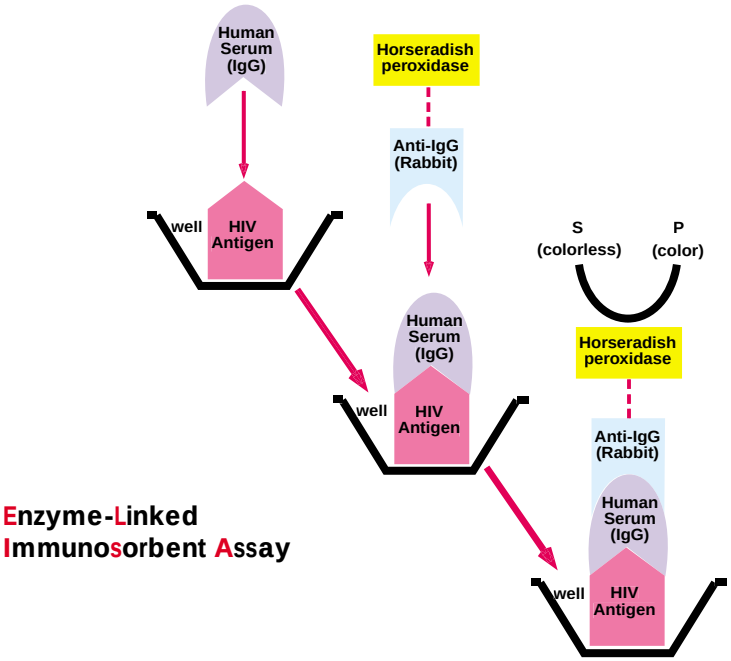
The immune system is the body's primary defense against infections by bacteria and viruses. If the human immune system is compromised, it does not have ability to fight off opportunistic infections, such as bacterial skin infections, viral induced skin cancers, and respiratory infections such as pneumonia.

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 immunoadsorbent 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 done 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



Enzyme-Linked Immunosorbent Assay

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) that is usually raised in rabbits or goats is added to the well that will bind to the primary antibody. The secondary antibody is cross-linked to the enzyme horseradish peroxidase. After washing the wells, a colorless solution containing hydrogen peroxide and a specific co-substrate (ABTS) is added which will develop color if the antibody is present.

This experiment is an ELISA simulation adapted to detect a hypothetical patient's circulating antibody directed towards a viral (HIV) antigen. Actual ELISA tests are generally done in microtiter plates made of a special type of plastic. The plates are transparent and contain many small wells, in which samples are deposited. This experiment uses an HIV Antibody Test strip with circles to simulate the wells.

Experiment Overview

Experiment Procedures

EXPERIMENT CONTENT OBJECTIVE

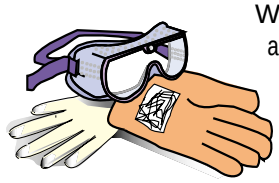
The objective of this experiment is to learn about basic concepts related to HIV infection, AIDS and the ELISA test, which is the initial screening test for the presence of the HIV virus.

WORKING HYPOTHESIS

If an individual has been exposed to the HIV virus and mounts an antibody response, then a positive color change will result when an ELISA test is performed.

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

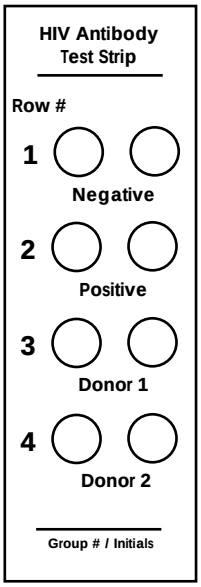


Wear gloves and safety goggles

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
3. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Conducting an ELISA Simulation

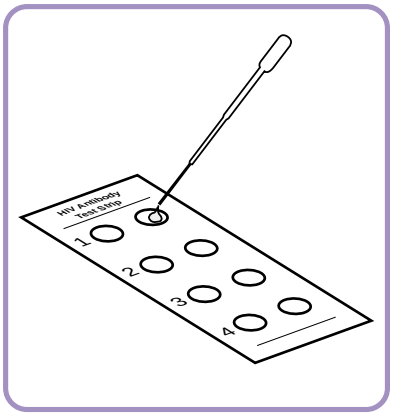


The circles on the HIV Antibody Test strip represent the wells of a plastic microtiter plate.

1. 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.

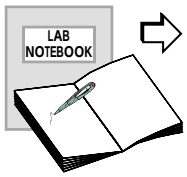
Experiment Procedures

Conducting an ELISA Simulation

Experiment Procedures

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.
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.

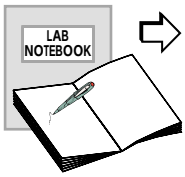
Critical Thinking and Hypothesis Development



For each Activity, record the following in your Laboratory Notebook or as instructed by your teacher.

1. What is the variable in this experiment?
2. What is the control in this experiment?
3. What could one change in the experiment if this experiment was repeated?
4. Write a hypothesis that would reflect a change.

Study Questions



Record the answers to the following Study Questions in your Laboratory Notebook or as instructed by your teacher.

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?

Notes to the Instructor

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines include Suggestions for Lesson Plan Content which can be adapted to fit your specific set of circumstances.

EXPERIMENT HINTS AND HELP

EDVOTEK experiments are easy to perform and are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties.

The EDVOTEK web site provides a variety of resources which are continuously being updated and added. If you do not find the answers to your questions in this section or at the EDVOTEK web site, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
web: www.edvotek.com
email: edvotek@aol.com

EDVO-TECH SERVICE
1-800-EDVOTEK
(1-800-338-6835)
Mon - Fri 9 am - 6 pm ET

Please have the following information:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

Suggestions for Lesson Plan Content

Instructor's Guide

This lesson plan outline, written by a teacher, can be used as a guideline for classroom discussion. Connections to the National Content and Skills Standards follow.

Purpose

To introduce students to the procedures involved in a clinical test for HIV.

Focusing Event

The experiment will simulate steps of a clinical laboratory test in which donor samples are screened for the presence of HIV.

Prerequisite Knowledge

Students should be familiar with the terms HIV, AIDS, Antigens, Antibodies and Immune System.

Experimental Skills

Students will become familiar with several terms used when discussing AIDS and some of the procedures involved in the ELISA detection test.

Materials

Components which are provided with this kit, and a list of requirements to perform this experiment are listed on page 3 of these experiment instructions. You may also wish to copy the figures which appear in the Background Information for use on an overhead projector.

Extensions

- Have students make a creative model of the HIV-1 virus to bring in and share with classmates.
- Have students conduct an on-line search to get the latest statistics on the epidemiology of HIV-1.
- Have student's research and write a report on the opportunistic infections that follow when the immune system is compromised after an HIV-1 infection.

continued

Suggestions for Lesson Plan Content

1. It is not necessary to explain in detail how the ELISA test works. You may want to use the simple diagram in the background information section to show how the parts bind to one another. As an introduction, the following may be explained:

    - AIDS is a disease caused by exposure to the Human Immunodeficiency Virus (HIV). This virus can be passed from person to person through direct blood to blood contact. Once in the body, HIV attacks and destroys portions of the immune system known as T Cells.

    - Without an efficiently working immune system, the body is unable to fight infections caused by bacteria and viruses. Patients with AIDS often become so ill that their body is unable to protect itself.

    - One of the first clinical screening tests for HIV is the Enzyme Linked Immunoadsorbent Assay, or ELISA. This test looks for antibodies the human body has made in response to the presence of the virus and in an attempt to fight it. The specific antibody the test looks for is called IgG.

2. Go over the procedure with the students. You may want to demonstrate to the students how much (one drop) of each solution to add to the circles on the test strip. Remind them that they will need to do 5 minute incubations.

3. Stress that the pipets must be rinsed between each step. You may wish to explain that in an actual clinical laboratory, a different "clean" pipet would be used for each individual sample to avoid contamination and false positive results.

4. Check each students' results. Emphasize the importance of a double test for accuracy.

5. After the experiment, bring closure to the activity.

    - Entertain questions from the students
    - Discuss how having a positive HIV test may or may not result in having AIDS
    - Discuss stereotypes and social issues relating to AIDS

Connections to National Content Standards

Instructor's Guide

1. Students will develop abilities necessary to do scientific inquiry.
   - Students' questions will be answered through conduction of a scientific investigation.
2. Students will develop an understanding through inquiry. Students will:
   - investigate a working hypothesis.
   - make careful observations.
   - interpret results correctly.
   - understand the difference between the experiment and the control.
   - identify variables.
   - predict experimental outcomes.
   - formulate explanations from evidence.
   - develop alternative explanations.
3. Students will use equipment, materials, and techniques for experimentation and direct investigation of phenomena.
   - Students will understand and interpret the diagnostic procedures involved in an ELISA test.
4. Students will develop an understanding of the immune systems role in fighting off viral infections.
   - Students will understand that the immune system produces a specific antibody towards the invading viral antigen.
   - Students will understand that the presence of the antibody against the antigen is determined by a laboratory test (ELISA) which is visualized by a color change.

Connections to National Skill Standards

In this experiment students will learn to use diagnostic procedures to determine if a person is infected by HIV. Analysis of the experiment will provide students the means to transform an abstract concept into a concrete experience.

Students will be able to:

1. Use scientific equipment such as calibrated pipets to develop accurate laboratory techniques.
2. Perform all the diagnostic steps accurately.
3. Make careful observations and record and interpret results.
4. Predict experimental outcomes.
5. Share and compare results obtained with other students.
6. Explain the implications of positive results.

Pre-Lab Preparations

Instructor's Guide

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.

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

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Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **"+" and "Donor 2"**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 07/01/03

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
CAS# 77-09-8 Highly flammable				

Section III - Physical/Chemical Characteristics

Boiling Point: For 1% solution	78° C	Specific Gravity (H_20 = 1)	.79
Vapor Pressure (mm Hg.)	44	Melting Point: -114°C 760mmHg & 261-263°C	
Vapor Density (AIR = 1)	1.6	Evaporation Rate (Butyl Acetate = 1)	2.7

Solubility in Water: Complete 100%

Appearance and Odor: Clear, colorless liquid, pleasant odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 12°C		3.3%	19%

Extinguishing Media: Use alcohol foam, dry chemical or carbon dioxide (water may be ineffective)

Special Fire Fighting Procedures: Wear protective equipment, SCBA

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources and flash back. Closed containers exposed to heat may explode, contact with strong oxidizers may cause fire. Emits toxic fumes in fire.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Heat, flame, or other sources of ignition. Sunlight and UV light
	Stable	X	

Incompatibility: Strong oxidizers, aluminum, alkali metals, acetyl chloride, strong acids

Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	x	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): May be harmful if inhaled, ingested, or absorbed by skin, causes eye irritation and mucous membrane irritation

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Headache, nausea, vomiting, dizziness, drowsiness, dermatitis

Medical Conditions Generally Aggravated by Exposure: Liver disorders, skin disorders, chronic irritation of mucous membrane

Emergency First Aid Procedures: Call physician. If swallowed, give large amounts of water. Induce vomiting. If inhaled, remove to fresh air. Give oxygen if have difficulty breathing. CPR if not breathing. Flush skin/eyes if contact is made.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear SCBA and protective equipment. Avoid all sources of ignition. Use absorbant material and place into container for proper disposal.

Waste Disposal Method: Observe all state and local regulations

Precautions to be Taken in Handling and Storing: Chemical splash goggles, chemical resistant gloves, NIOSH/MSHA approved respirator safety shower, mechanical exhaust, keep container tightly closed.

Other Precautions: Avoid contact

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved

Ventilation			
	Local Exhaust Yes	Special	
	Mechanical (General)	Other	

Protective Gloves: Chemical resistant

Eye Protection: Chemical resistant splash goggles

Other Protective Clothing or Equipment:

Work/Hygienic Practices:

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Substrate (S-70)

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: **07/01/03**

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
CAS # 1310-73-2				

Section III - Physical/Chemical Characteristics

Boiling Point		Specific Gravity (H_20 = 1)	2.13N/A
Vapor Pressure (mm Hg.) >18mm at 20°C 3mm at 37°C		Melting Point	318°C
Vapor Density (AIR = 1)	>1	Evaporation Rate (Butyl Acetate = 1)	

Solubility in Water: Soluble

Appearance and Odor: Clear, colorless liquid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL

Extinguishing Media: Use exstinquishing media appropriate to surrounding area, do not use water

Special Fire Fighting Procedures: Wear SCBA and protective clothing to avoid exposure to skin and eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Absorbs CO2 from air
	Stable		

Incompatibility: Strong oxidizing agents, strong acids, organic materials

Hazardous Decomposition or Byproducts: Nature of decomposition products unknown

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur		

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic):

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea, and vomiting

Medical Conditions Generally Aggravated by Exposure:

Emergency First Aid Procedures: Immediately flush eyes or skin w/ copious amounts of water for at least 15 minutes, remove to fresh air. Wash out mouth if swallowed.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear SCBA, heavy rubber gloves, and boots. Ventilate area after complete.

Waste Disposal Method: Neutralize with water, flush down drain. Observe all federal, state, and local environmental regulations

Precautions to be Taken in Handling and Storing: See "+" and "Donor 2"

Other Precautions: Wash up thoroughly

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA - approved respirator

Ventilation			
	Local Exhaust Yes	Special	
	Mechanical (General)	Other	

Protective Gloves: Yes

Eye Protection: Chem. resistant splash

Other Protective Clothing or Equipment: Chemical resistant

Work/Hygienic Practices: