96-0342-S Rev AS

Neo/SCI Student's Guide

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New ideas for teaching science

ELISA:HIV/AIDS Test Simulation

Objectives

- ***Learn*** **about basic immunology concepts**
- ***Learn*** **about HIV infection and AIDS**
- ***Understand*** **how the ELISA for HIV screening works**
- ***Observe*** **simulated ELISA antigen/antibody reactions**
- ***Analyze*** **ELISA test results**

Background

AIDS is an infectious disease that is caused by a virus called HIV. AIDS is short for **A**cquired **I**mmune **D**eficiency **S**yndrome:

Acquired - received it from something or someone else
Immune - body's resistance to disease, body's defense system
Deficiency - lacking, not working properly
Syndrome - a group of signs or symptoms that together usually indicates a particular disease

HIV is short for **H**uman **I**mmunodeficiency **V**irus:
Human - people
Immunodeficiency - the body's defense system is not working
Virus - an organism that causes disease

The Human Immunodeficiency Virus (HIV) attacks and destroys the body's helper T cells (CD 4 cells), the very cells the body uses to defend itself against infection.

Effectively, HIV destroys our body's defense (immune system). It is a progressive disorder that eventually prevent the body resisting attack by other diseases, known as opportunistic diseases, such as Kaposi's Sarcoma (skin cancer), Pneumocystis Carinii Pneumonia (PCP), Thrush, Cryprococcal meningitis, TB, and others, finally ending in death.

It is currently estimated that there are over 36 million adults and children in the world living with HIV/AIDS. Of these, 25 million, more than 70% of the world total, live in Sub-Saharan Africa. South Africa has more people living with HIV/AIDS than any other country in the world. To date there is no effective vaccine available to protect against HIV infection. Although certain medications are available which slow down the progression of the disease, there is no cure. The best method to combat the spread is by education, prevention, behavior changes and responsible management and treatment of the disease and the opportunistic infections.

Where did AIDS originate?

AIDS is believed to have originated in Central Africa and was first recognized in 1981 by doctors in the US. Doctors were alerted to the syndrome when they noticed an increase in the number of patients presenting with what had, up until then, been regarded as rare disease and ones to which the body was normally resistant. The increase in conditions such as pneumonia caused by Pneumocystis Carinii, cancer caused by Kaposi's Sarcoma, and mouth sores led them to believe that these patients' immune systems were compromised (weakened).

Soon these same symptoms were noticed in patients in other parts of the world, among various population groups, including hemophiliacs who had received numerous blood transfusions, and intravenous drug users. It soon became clear that they were dealing with a pandemic and set to work to find what was causing it.

In 1983, French scientists isolated a retrovirus, HIV. Later in the same year the virus was isolated in the US.

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HIV is spread when bodily fluids containing the virus are passed from person to another. There are three main modes of transmission: sexual transmission, blood-to-blood transmission and mother to child transmission.

How does HIV cause AIDS?

HIV causes AIDS by overpowering the body's immune system (defense system) so that is helpless and cannot fight infections. It does this by destroying the immune system's helper T cell (also known ad CD4 cells.)

There are four phases of the disease and these relate closely to the HIV's activity and progress in the body at the time:

Phase 1 – Shortly after infection the body produces antibodies to the HIV virus and the individual experiences flu-like symptoms such as fever, headaches, joint and muscle pains, a skin rash or swollen glands. The symptoms disappear after a week or two and the individual feels well, looks perfectly healthy and is often unaware that he has been infected with the virus.

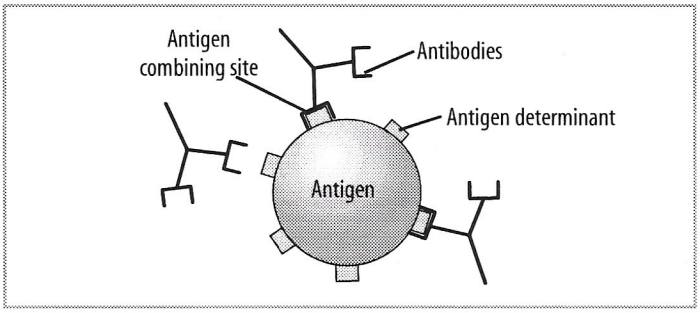
Phase 2 – The clinically latent phase when HIV antibodies can be detected in the blood but the infected individual is free of symptoms. This latent phase can vary from a few weeks to 10 and more years, depending on the person's state of health and lifestyle.

Phase 3 – The virus is starting to get the upper hand and the immune system is weakening. Certain sign and symptoms alert the doctor the onset of AIDS. The individual suffers from opportunistic infections that are not too severe at this stage; however, they become more frequent and persistent. This phase heralds the real onset of AIDS.

Phase 4 – Full blown AIDS is characterized by low CD4 count and severe opportunistic infections, Kaposi's Sarcoma (pink skin lesions), Pneumocystis Carinii pneumonia (PCP), TB, Cryptococcal meningitis, Candidiasis mouth thrush, weight loss and wasting syndrome.

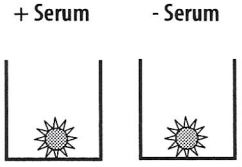
The ELISA test

The ELISA test is based on the principle that antibodies bind to their specific antigens. The ELISA test detects the presence or absence of HIV antibodies in a serum sample thereby indirectly testing for the HIV virus. The patient's blood is added to the HIV antigen. If HIV antibodies are present, they will bind to the HIV antigen and the amount of antibody present can be measured by an enzymatic color reaction.

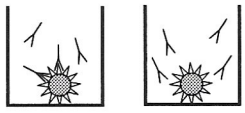


The test is performed in a micro-well plate as follows:

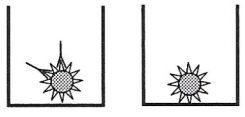
1. Purified (or a genetically engineered replica of the HIV virus is fixed to the bottom of each well or coated on a round bead that is placed in the well.

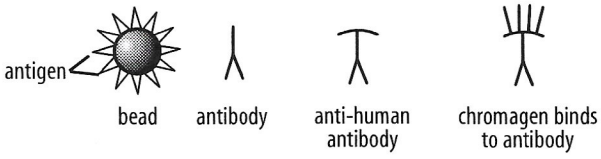


2. Patient's serum is added to the well containing the HIV antigen and is incubated for a few minutes to allow HIV antibodies in the patient's serum to combine with (bind to) the HIV antigen fixed to the well or the bead.



3. The liquid contents of the well are then removed and unbound serum is discarded. The wells are washed to remove all traces of unbound material. Bound HIV antibodies remain fixed to the HIV antigen on the well or bead.





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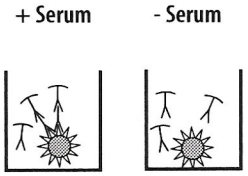
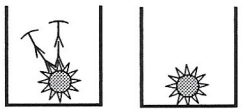
At this point you cannot see or detect the antibody/antigen complex. A **conjugate** consisting of anti-human antibodies linked to an enzyme triggers a reaction with a color producing substance called **chromagen**. The resulting color can be quantified with a spectrophotometer.

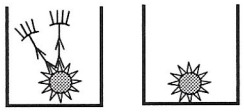
4. A conjugate is added to the well and incubated again. The conjugate's anti-human antibodies will bind to any human antibody present, and will also be fixed to the well or bead.



5. The liquid in the well is once again discarded and the wells are washed to remove any unbound material. A complex of HIV antigen/HIV antibody/enzyme linked conjugate will remain fixed only if HIV antibodies are present.



6. Chromagen (color producing agent) is then added. It reacts with the enzyme linked to the conjugate to produce a color that signals and amplifies even the smallest amount of antibody present. If there is no HIV antibody/enzyme complex in the well there will be no color.



7. The color is then read using a spectrophotometer. This allows the quantity of antibodies present to be measured. The color can also be read visually by comparing it to a standard color level. The intensity of the color produced is directly proportional to the amount of antibodies present in the patient's serum- in other words, the more color, the more HIV antibodies.

Known negative and positive sera are always run with the test as controls to ensure correct results. If the patient's blood tests positive with the ELISA test, further testing is performed using the Western Blot method to confirm the result. The accuracy of the combined ELISA and Western Blot tests is greater than 90%.

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The Body's Defense Against Infection

I

Barriers

Skin **Mucous Membranes**

Physical barriers preventing foreign organisms (pathogens) entering the body.

II

Non-Specific Internal Response

When physical barriers are broken and infectious organisms (pathogens) pass through and enter the body.

Inflammation

Extra blood flows to infected tissue site. It becomes red, hot, swollen and painful.

Phagocytosis

White blood cells,mainly the neutrophils and the monocytes (macrophages) ingest (swallow) the pathogens and destroy them.

Fever

Body's temperature is raised. Heat slows down the activity of bacteria and viruses allowing the body's own defense cells time to act.

Antimicrobial Effects

of body secretions eg. Hydrochloric acid of gastric juice, acidity of saliva, enzyme action, etc.

III

Specific Immune Response

Immunity means the body's ability to protect itself against, and build a resistance to, specific foreign substances (antigens) causing disease.

White Blood Cells

Monocytes (Macrophages)

Phagocytes that link the **non-specific** and the **specific** responses. They ingest pathogens, break them up and deposit fragments of them on the macrophage surface for the T cell's to recognize as antigens (foreign substances).

Lymphocytes

B Lymphocytes form Antibodies

Antibodies only react with **the specific antigens** that stimulated their formation. They are secreted into the blood and act by: a) binding to their **specific antigen** and b) activating the **complement system**. These actions **destroy antigens** by causing:

- Agglutination (clumping together)
- Lysis (rupturing the cell wall)
- Opsonisation (Making it susceptible to phagocytes)
- Neutralization (of toxins)

Future Immunity (Resistance)

Some **antibodies** are stored as **"memory antibodies"** that react immediately against **specific antigens** if they enter the body again.

T Lymphocytes become Sensitized T cells

Act against specific antigens in the body. There are 3 types of T cells: **Killer T cells** - attach to and destroy infected cells. **Helper T cells** (CD 4 cells) - stimulate B lymphocytes to form antibodies and killer T cells to destroy infected cells. **Suppressor T cells** - switch off the Immune Response.

Future Immunity (Resistance)

Some **T cells** are stored as **"memory T cells"** and will react immediately against **specific antigens** if it attacks again.

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ACTIVITY 1

Simulated HIV Test

Overview

You are going to perform a simulated HIV ELISA test on nine patients. You will be provided with a scenario for each patient.

- Read through each scenario and discuss it with your group
- Try to predict which of the patients would test positive and negative and discuss why you think so.
- Perform the test and check your results with your teacher. Answer the questions in the Analysis section and discuss your answers with your group.

Safety & Disposal

This lab investigation includes simulated non-biological solutions and is used to mimic the actual steps of an HIV ELISA test. Because the test is simulated and does not use any biological solutions you cannot be infected with HIV/AIDS or any other infection from the contents of this kit. However, be sure to use proper chemical laboratory protocol and follow all of the safety instructions included.

Be sure to always wear safety goggles, gloves and a lab apron to protect your eyes and clothing when working with any chemicals.

Dispose of any waste materials and clean up your work area as directed by your teacher.

Be sure to always wash your hands before leaving the laboratory.

Patient Scenarios

Jan

Lynn

Baby Elizabeth

Roger

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Bob

Tony

Richard

Tania

William

What you need

Per student

Gloves

Goggles

Apron

Per group

1 Microwell strip

1 Microspatulas

1 Plastic pipets

12 Glass beads coated with simulated HIV antigen

Marking pencil

Per class

Simulated anti-human antibody enzyme linked conjugate, 10mL

Simulated chromagen, 10mL

Simulated patients sera, 10mL

Simulated negative control serum, 10mL

Simulated low positive control serum, 10mL

Simulated high positive control serum, 10mL

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What to do...

Step 1 – HIV antigen

Your teacher will provide you with two 8-microwell strips. Label the wells #1-12. Using a microspatula, add one glass bead coated with coated with simulated HIV antigen to each of wells 1-12 of the microwell strip.

Step 2 – Sample diluent

Using a pipet, add 2 drops of water to each of the 12 wells.

Step 3 - Controls

Add 2 drops of each control as follows:

Negative control to well #1
Low positive control to well #2
High positive control to well #3

Step 4- Patients' sera

Add 2 drops of each patient's serum as follows:

Jan to well #4
Lynn to well #5
Baby Elizabeth to well #6
Roger to well #7
Bob to well #8
Tony to well #9
Richard to well #10
Tania to well #11
William to well #12

Step 5 – Incubate

Incubate your microwell strip for 3 minutes at room temperature.

Step 6 – Add Conjugate

Add 2 drops of Conjugate to the sera in each of wells 1-12.

Step 7- Incubate

Incubate your microwell strip for 3 minutes at room temperature.

Step 8- Chromagen

Add 2 drops of Chromagen to each of wells 1-12.

Step 9 – Read Control Results

Control Sera:

Negative:	Colorless or light yellow
Low Positive:	Light orange
High Positive:	Dark red

Step 10 – Interpret Patients' Results

Positive:	Color greater than or equal to low positive control
Negative:	Color light yellow or less than low positive

Record your results and observations in the Data Table.

Step 11 – Cleaning

Discard well contents into waste container. Rinse the microwell strips thoroughly with water. Rinse microspatulas under running water. Rinse pipets by sucking water up and discarding three times. Air dry materials before packing them away.

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Data Table

Well Number	Patient	Result	Observations/Comments
1	Negative Control		
2	Low positive Control		
3	High Positive Control		
4	Jan		
5	Lynn		
6	Baby Elizabeth		
7	Roger		
8	Bob		
9	Tony		
10	Richard		
11	Tania		
12	William		

Questions

1.

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Going Further

Demonstrate how scientific knowledge is applied to make blood safe for transfusion with particular emphasis on how your local blood bank goes about it. Take a survey to find out if the general public aware of it.

HIV is a retrovirus. What does this mean and how does it react when it enters the body? Demonstrate your findings with reference to the Immunodeficiency virus from the time it enters the body to the development of AIDS.

There are over 36 million people living with HIV/AIDS in the world. Since there is currently no vaccine to prevent infection and no medicine to cure HIV infection, we need to know how people get infected and how we can prevent the spread of HIV/AIDS. Investigate how HIV is spread and use your findings together with your knowledge, particularly with the body's first line of defense to demonstrate ways in which we can protect ourselves and others against infection and prevent the spread of HIV.

Learn and Read More About It

Level 4: Virus Hunters of the CDC
by Joseph B. McCormick, M.D. and Susan Fisher-Hoch, M.D.

Virus X
by Frank Ryan, M.D.

Inside AIDS: HIV Attacks the Immune System
by Conrad J. Storad

The Virus Invaders
by Alan E. Nourse

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Neat Websites

HIV/AIDS Information Center
Journal of the American Medical Association
http://www.ama-assn.org/special/hiv/hivhome.htm

Centers For Disease Control & Prevention - AIDS Clearinghouse
http://www.medpatients.com/Health Resources/cdc-aids.htm

Provides information on blood banking
Http://cer.hs.washington.edu/John/blbnk.htm

Information on blood and blood typing from Red Cross
http://www.redcross.org/oh/northernohio-blood/bloodtype.html

National Heart Lung, and Blood Institute
http://www.nhlbi.nih.gov/nhlbi/nhlbi.htm