Hunting the Immune Villain©

Using ELISA to diagnose HIV

Student Guide

BioBus Educational Programs

Version 1

Version 1

STUDENT CASE BRIEF

WHAT IS A VIRUS?

A virus is a **submicroscopic** particle which infects or invades the cells of a biological organism. Viruses cause many common infections and diseases ranging from the common cold (e.g. *rhinoviruses*) to acquired immunodeficiency syndrome (human immunodeficiency virus). A virus cannot grow or reproduce outside of its host, a living cell. Once a virus invades its host, it uses the host cellular machinery (**enzymes**, proteins, etc.) to replicate its genome and to produce more virus particles. A virus may produce exact duplicates of itself or it may produce copies with genetic mutations or errors. The more a virus mutates, the harder it is for a host to treat or fight the viral infection.

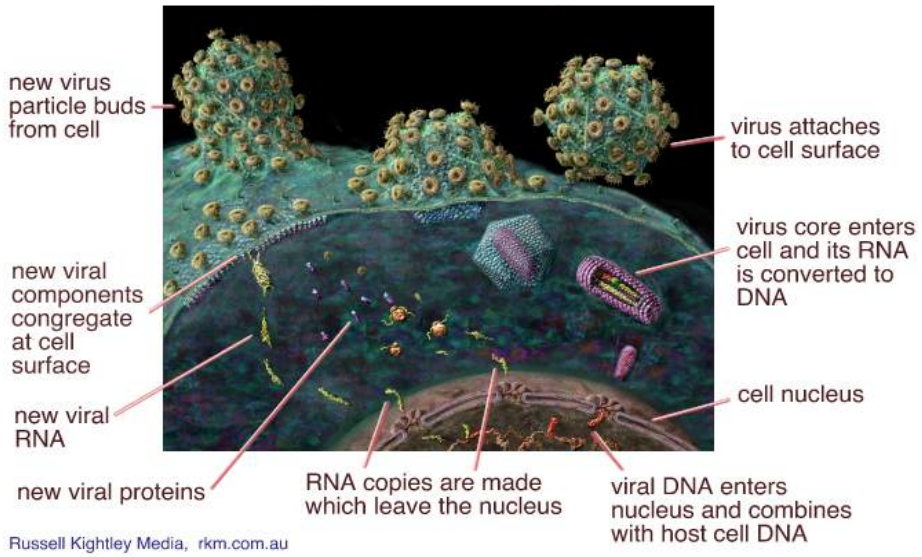
Viruses may contain either DNA or RNA as their genetic material. Human immunodeficiency virus, or HIV, is an RNA **retrovirus**. This means that HIV must first convert its RNA genome into DNA and then use the DNA as a template for making new viruses.

Virus

(vI-rs) – A submicroscopic particle which infects or invades the cells of a biological organism.

DNA → RNA → Protein

▲ **Retrovirus.** A retrovirus must first convert its RNA genome into DNA before it can be used as a template to make viral proteins.



◄ **Life cycle of HIV.** First the virus attaches to the surface of a cell. The viral core is then inserted into the cell and the RNA genome is converted into DNA. The viral DNA then enters the host nucleus and inserts itself into the host genome. RNA copies are then made which are translated into viral proteins by the host cell. The new viral components assemble at the cell surface and bud off from the host cell creating a new viral particle ready to infect another cell. Photo source: http://www.mcb.uct.ac.za/HIVannot.jpg

DISCOVERY OF HIV AND AIDS

As is the case with many viruses, the disease which HIV causes, Acquired Immune Deficiency Syndrome, or AIDS, was discovered before the virus itself. AIDS is a syndrome in which a person's **immune system** starts to fail, making them susceptible to many **opportunistic infections**. Because people with AIDS have a compromised immune system, these opportunistic infections now become life-threatening. The AIDS epidemic was first recognized by the US Centers for Disease Control and Prevention (CDC) on June 5, 1981. It wasn't until two years later that scientists demonstrated that HIV causes AIDS. Today, more than 25 million people have died as a result of HIV/AIDS.

Opportunistic Infection

(ā-pr-tŭ-nis-tik in-fek-shn) – An infection which does not normally cause disease in a person with a healthy immune system, but can cause disease in a person with a suppressed immune system.

Where did HIV come from? The origin of HIV-1, the most common form of the virus, is now clear. Evidence for the origin of HIV-1 was obtained by Beatrice Hahn and colleagues. Her evidence shows that a chimpanzee form of the virus, a **S**imian **I**mmunodeficiency **V**irus (SIV), most likely jumped from chimpanzees to humans in the African country of Cameroon sometime in the early 20th century. The HIV species jump was most likely made possible through the consumption of bush meat and the keeping of infected animals as pets. Once in humans, the SIV virus likely mutated into the current form of HIV-1.

HIV TRANSMISSION

HIV can enter the body whenever there is an exchange of bodily fluids with an infected person. HIV is found in blood, semen, vaginal fluid, breast milk, saliva and tears. The most common way of contracting HIV is by having unprotected sex (oral, vaginal, or anal) with an infected person. The use of latex or polyurethane condoms can greatly reduce the risk of acquiring or transmitting HIV. However, for condoms to provide this protection they must be used consistently and correctly. Lambskin or natural membrane condoms do not provide protection against HIV because they can contain natural pores through which the HIV virus can pass.

There are three other common ways in which HIV is spread. HIV can be transmitted through needles, affecting for example health care providers who have been stuck by an infected needle, or intravenous drug users who have used a dirty (previously used) needle from an infected individual. HIV can also be transmitted between a mother and child either during pregnancy, birth or breastfeeding. However, if a mother is treated for HIV during her pregnancy this risk can be greatly reduced. Finally, in the 1980's, blood transfusions were also known to transmit the virus, but in the US this is no longer a problem because blood is now screened for HIV before it is used.

There are many misconceptions about how HIV is transmitted. HIV cannot be acquired by insect bites, such as mosquitoes, or other routes such as air, environmental surfaces, or water. There are no known cases of HIV being transmitted through casual kissing. There is one documented case in which a person acquired HIV through open mouth kissing, but in this case the person had an open sore in his/her mouth, allowing the virus to enter the blood system. Finally, biting has been documented to transmit HIV, but only in cases with severe tissue damage. There have been many reported cases in which a bite *did not* transmit HIV.

HIV CAUSES AIDS

HIV attacks certain cells of the immune system, the most known of which are CD4 T cells (also known as T4 cells and Helper T cells). T cells represent an important line of immune defense, helping the body fight off disease and infection. Interestingly, once the body has been infected with HIV, the virus can stay dormant for years. Upon initial **infection**, the body mounts an **immune response** and the infected person will appear to recover, usually feeling healthy. This healthy feeling can continue for many years; as

Fact Files

First Case of AIDS

The first reported case of AIDS was in an African Bantu man from Leopoldville, Belgian Congo. He died of AIDS in 1959. Scientists have used his blood sample to map the evolution and spread of the HIV virus worldwide.

▲ Cross-section of an HIV particle. Photo Source: US Department of Energy

Fact Files

HIV life cycle

The HIV life cycle lasts about 1.5 days. Since HIV does not have proofreading enzymes to correct genetic mistakes, HIV frequently mutates. While many of the mutations generated are unfavorable, resulting in rapid destruction, sometimes the mutations are favorable, allowing the virus to elude the host's immune system and antiretroviral drugs.

long as the viral levels remain low and there are sufficient T4 cells circulating in the blood stream. However, as viral production increases, and these new viruses go on to infect more T4 cells, the body begins to lose the battle against HIV. When T4 cells "shed" or release new viruses, they are often killed. Eventually, the rate at which T4 cells are killed surpasses the rate at which new T4 cells are generated, leaving the body incapable of fighting off other infections. The person is now susceptible to opportunistic infections. Once an individual's T4 cell count is at or below 200 cells/µL of blood (normal T4 counts are around 1000 cells/µL), they are often officially diagnosed with AIDS.

SIGNS, SYMPTOMS AND DIAGNOSIS

Diagnosing HIV can be difficult because the symptoms of HIV are often vague and similar to many other diseases. Some signs and symptoms of HIV include fatigue, swollen lymph glands, night sweats, rapid weight loss, frequent bruising and constant fever. Many of these are common symptoms of a cold or the flu. The only way to confirm HIV infection is through a blood test that detects **antibodies** made by the immune system to fight the virus.

Frequently, the test used to detect the presence of HIV antibodies is an **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay or **ELISA** (see next page). This is the test you will be performing. If HIV antibodies are present in the sample, the test will read positive. If there are no HIV antibodies in the sample, the test will be negative. However, a negative result does not mean that the person being tested is HIV-negative. It generally takes about 2 to 8 weeks from the time a person becomes initially infected with the virus to when then their body begins to produce antibodies to fight the infection. This is called the "window period." It is important to wait until after this window to get tested. Therefore, physicians usually recommend a retest for any person receiving an initial negative test result.

TREATMENTS

Most of the time viruses (like flu or cold viruses) cannot be treated and don't need to be treated because the human body's immune system can eliminate most viral infections in a few weeks or less. Remember, antibiotics do not treat viruses, they treat bacterial infections. Because AIDS is such a devastating disease, drugs have been developed to treat it. However, it has been difficult to specifically target HIV because the virus mutates rapidly, changing its appearance to the immune system.

AIDS patients are usually treated with antiretroviral drugs. Unfortunately, none of these drugs cure HIV infections or AIDS; they only suppress the virus. It is very common for HIV patients to develop resistance to one or more of these drugs. Therefore, HIV patients are generally treated with many different types of drugs, called a cocktail, rather than just one medication. Pharmaceutical companies and scientists are constantly trying to develop more effective treatments, and ultimately a cure for HIV/AIDS.

Insect Bites Do Not Transmit HIV?

To date there have been no documented cases of a person contracting HIV from an insect bite, even in populations where HIV is widespread. Insects do not get infected with HIV. When an insect, such as a mosquito, bites, it injects its own saliva which contains chemicals to prevent blood clotting. It does not inject blood gathered from a previous bite.

Fact Files

T Cell Padawans

Only about two percent of all T cell precursors (or Padawans for Star Wars fans) actually pass the tests to become mature T cells!

Go Online!

For: HIV Quizzes
Visit:
http://www.avert.org/hivquiz.htm

INTRODUCTION TO IMMUNOLOGY

Antibodies are proteins produced by white blood cells in response to foreign materials. Foreign materials, known as **antigens**, can be infectious agents (e.g. viruses or bacteria) and environmental materials (e.g. pollen.) Antigens can be proteins, carbohydrates, or nucleic acids. Antibodies, produced in response to antigens, bind antigens so that **macrophages** can remove them from circulation. An antibody binds to an antigen due to its specific chemical structure (think of a lock and a key). Each antibody has two antigen binding sites (see diagram at right).

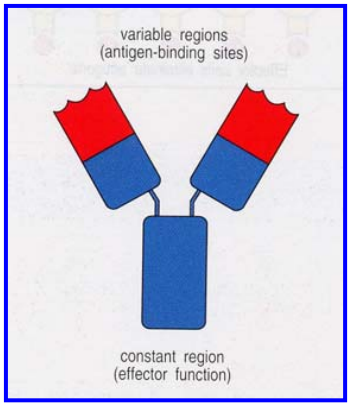


Diagram of an antibody.

ELISA

The **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay (ELISA) is used to diagnose a variety of diseases and conditions including tumors, pregnancy, narcotic usage, rubella, herpes, hepatitis, measles, and mumps. The ELISA for this experiment is designed to detect an antibody directed at a specific antigen: the HIV virus (you will use *simulated* antigens in your experiment). The ELISA uses specific antigens found on the surface of HIV to detect the presence of antibodies being produced to combat the virus.

Antigen

(an-ti-jn) – Any foreign substance introduced into the body.

REFERENCES

Janeway and Travers. (1997) *Immunology* (Third Edition). Churchill Livingstone.

Stine, Gerald J. (2002) *AIDS Update 2002*. Upper Saddle River, New Jersey: Prentice Hall.

United States Centers for Disease Control and Prevention

GLOSSARY OF TERMS

Antibody - A protein made by the immune system that recognizes and binds to a particular antigen to prevent that antigen from harming the body.

Antigen - Any foreign substance introduced into the body.

Enzyme - A protein which catalyzes a biochemical reaction.

Enzyme-Linked ImmunoSorbent Assay (ELISA) - A scientific technique which measures antibody levels.

Immune - Not susceptible to a particular infection, usually because the body produces antibodies which recognize the antigen.

Immune Response - How an organism recognizes and fights against antigens.

Immune System – The machinery an organism has to fight off foreign invaders.

Immunology - The study of the immune system and its responses.

Infection - The result of an antigen attacking an organism.

Infectious Disease - A disease which can be easily passed on to many organisms within or between species.

Integrase – An enzyme produced by a virus that enables genetic material to be integrated into the DNA of the infected cell.

Inflammation - The body's response to an infection or injury commonly characterized by pain, redness, heat and swelling.

Lymphocyte - A type of white blood cell which aids the immune system in fighting an infection, includes T-cells and B-cells.

Macrophage - A cell in the immune system which fights infections by engulfing antigens and breaking them apart.

Opportunistic Infection – An infection that does not normally cause disease in a person with a healthy immune system, but can affect people with a suppressed immune system.

Protease – An enzyme that assists in the breakdown of proteins into short peptides and amino acids.

Retrovirus - A virus whose RNA genome must first be converted to DNA before it is integrated into the host genome.

Reverse Transcriptase – An enzyme that can transcribe RNA into DNA.

Specificity - The condition of belonging to a particular group.

Submicroscopic – Particles of matter that cannot be seen under most optical microscopes.

Symptom - Something which indicates the presence of a particular condition, disease or disorder.

Transcription – The synthesis of an RNA copy from a sequence of DNA.

Translation – The synthesis of a peptide or protein from an mRNA sequence

Vaccine - A preparation of a weakened or killed form of a particular antigen that is administered to produce immunity to that antigen.

White Blood Cell - A component of blood which helps to defend the body against antigens

Name: _____

BIOBUS LABORATORY NOTEBOOK

TITLE OF EXPERIMENT:

OBJECTIVE:

PREDICTION:

EXPERIMENTAL PROCEDURE:

1	**2**	**3**	**4**
5	**6**	**7**	**8**

BIOBUS LABORATORY NOTEBOOK CONTINUED

EXPERIMENTAL PROCEDURE CONTINUED:

RESULTS:

1	**2**	**3**	**4**
5	**6**	**7**	**8**

CONCLUSION:

Petri Dish Template