Cornell Institute for Biology Teachers

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Title: **HIV Testing**

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Appropriate Level: Regents, Honors, or AP Biology.
The hands-on experiment is extremely easy to carry out. To increase the level of the lab, more detail on immunology and the life cycle of the HIV virus can be included.

Abstract: In this experiment students will perform an **Enzyme-linked Immunosorbent Assay (ELISA)** simulation to see how it is used in the diagnosis of HIV infection. The lab is a simulation and does not use HIV antigen or human blood samples; however, it is otherwise a truly accurate depiction of the technique. Students will process samples from two different "patients" to determine whether they have been infected with HIV. We recommend that you combine this activity with the CIBT activity entitled "AIDS Transmission" and a discussion of "safe sex" activities. Several thought provoking questions have been included at the end of the lab. You may want to require student essays or ask the students to approach these questions using the CIBT Bioethics Activity. The CIBT lab, "'The Cocktail': A Magic Potion Against HIV?" is a good way to discuss treatment.

Time Requirement: The activity fits nicely into an 80 minute class period. While the color reactions are developing, students can do the "AIDS Transmission" activity, the "'The Cocktail': A Magic Potion Against AIDS" computer activity on drug design, or have a discussion about the biology of the HIV virus, ethical issues, etc.

Additional Teacher Information

An **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay (**ELISA**) is used to test for infection by HIV. A positive test indicates that antibodies against the HIV virus are present in the blood. If antibodies are present in the blood sample, they will bind to antigens that have been immobilized in a plastic reaction tube. To determine whether antibodies have been bound by the antigen, a 2° antibody is used. The 2° antibody is produced in an animal (for example, a rabbit) in response to injection with human antibodies. The 2° antibody is covalently attached to an enzyme, like alkaline phosphatase, to make it easy to determine whether or not 2° antibody is present. If HIV antibodies are present, they will bind to the HIV antigen. When **enzyme-linked** anti-human antibodies are added, they will bind only if human anti-HIV is bound. To determine whether or not enzyme-linked anti-human antibodies are present, a substrate is added that changes color when cleaved. In this case the substrate, 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS) turns a blue-green color when cleaved. A positive test result is a change from a colorless solution to blue-green.

Since the specificity of the test is based on binding, it is important that each step be followed by careful washing procedures. During the washes, unbound materials are removed from the plate and only the bound components will remain behind. The washes are carried out in PBS and PBS/Tween 20. PBS is a is a buffer that will maintain the pH within a biological range. Tween 20 is a detergent that is added to enhance the effectiveness of the washes. Tween 20 washes are always followed by PBS washes to remove traces of detergent that might denature the antibodies that will subsequently be added.

The plates included in the kit were initially treated with antigen. (albumin) Following treatment with antigen, they were treated with PBS/ 3% milk to bind all remaining binding sites in the tubes. If protein diffuses from these spots at any point during the procedure, it is possible that antibody proteins will bind to the tube based on non-specific binding to plastic rather than by specific binding to antigens. If non-specific binding occurs, a "false positive" result will be obtained. Therefore during some steps, PBS / 3% milk is used to wash the plates to ensure that plastic tubes remain coated with protein at all times.

To reduce the chance of contamination, stress that students should not use the same forceps in different wells. Students may also be tempted to pop bubbles formed in each well with the droppers which may also cause contamination and a false test result. Since a false positive test is possible even in a clinical lab, it should be stressed to the students that *any positive test is routinely repeated to be certain of the result*. A positive ELISA test is also routinely confirmed by a Western Blot based procedure.

Preparation Time Required

You should arrange for the materials several weeks before you plan to do the activity. Once the materials arrive, it will take about one hour to make the PBS, PBS/Tween, PBS/3% milk and to set out the materials for the lab activity.

Materials

per team of students

- 1 strip of reaction tubes (4 tubes / strip)
- positive control filter paper disks (labeled "P")
  - store at 4°C
- negative control filter paper disks (labeled "N")
  - store at 4°C
- patient #1 filter paper disks (labeled "1")
  - store at 4°C
- patient #2 filter paper disks (labeled "2")
  - store at 4°C

per class (sufficient for 12 teams of students)

- 20 ml PBS / albumin solution - store at 4°C
- 1 tube with 0.25 ml 3% hydrogen peroxide (fresh)
- 1 foil wrapped substrate tablet (Sigma # A-9941)
- 50 ml substrate buffer solution
- 1 tube with 0.5 ml Tween 20 detergent
- 1 tube with 2.25 gram powdered non-fat milk
- 1 tube containing enzyme-linked animal antibodies (Sigma # A1949) - store at 4°C

Teacher should provide:

- fine point permanent marker pen
- transfer pipettes (5 per reagent set - tape a small test tube to each reagent bottle. Between uses, the transfer pipette can be kept clean by slipping it into the tube and therefore can be shared by a number of students.)
- forceps (4 per pair of students)
- paper towels (2 per pair of students)
- latex gloves if you want to emphasize the potential infectious nature of the samples

Solutions prepared by teacher:

- 1 liter PBS (dissolve 8 g NaCl, 2 g KCl, 2 g KH_2PO_4, 12 g Na_2HPO_4 in 1 liter of distilled water)
- 3% milk in PBS - Add 75 ml PBS to the tube from the kit that contains 2.25 g dried milk
- PBS/ Tween (Add ~200 ml of PBS to the tube containing 0.2 ml Tween 20 detergent, mix and add to 290 ml PBS)

- enzyme labeled antibody solution - **Make just before use**. Add 1ml of PBS / Milk to the tube labeled 2° antibody and invert ten times to mix (DO NOT SHAKE). Then add to 19 ml of PBS / Milk to make a total of 20 ml.

- Substrate Solution - **Make just before use**. Add 1 ml of substrate solution to the tube labeled peroxide. Mix and add back to the remaining solution in the tube labeled substrate solution. Remove the substrate tablet from the foil wrapping and add to the solution. Invert until fully dissolved.

- As a positive control of the detection reagents and to sensitize the students to the color they should expect for a positive result, transfer 5 ml of substrate solution to a test tube, add 5-10 drops of the enzyme labeled antibody solution in PBS milk. If all is well, in a few minutes the tube should turn green.

To coat the plates: (Either you can do this or if students have enough lab time, they can do it.)

Fill each well of each plate with PBS/ albumin and incubate in the refrigerator for at least one day. After 1 - 7 days, empty the PBS albumin into the sink and rap the plates 3 times briskly over paper towels to remove remaining liquid. Fill wells with PBS / Milk and incubate 1 hour at room temperature (or overnight at 4°C). Rinse and rap four times with PBS / Milk. The plates are now at the appropriate stage to use with the attached student protocol.

Special Notes:

A virtual lab computer site can be viewed by students after completing this lab. The URL is: **http://www.hhmi.org/grants/lectures/vlab/**

A second site of interest is **http://www.cellsalive.com**. (This site deals with HIV infection as well as immunity.)

Answers to Pre-lab questions:

2. List and describe four symptoms/ conditions commonly associated with AIDS.

   (1) Kaposi's syndrome
   (2) susceptibility to pneumonia and other diseases
   (3) dementia
   (4) death

3. List two ways that you can protect yourself against infection by the HIV virus.
   (1) abstinence
   (2) use of a condom and other "safe sex" practices

4. What is an antibody and why are antibodies important?
   One of millions of blood proteins, produced by the immune system, which specifically recognizes a foreign substance and initiates its removal from the body.

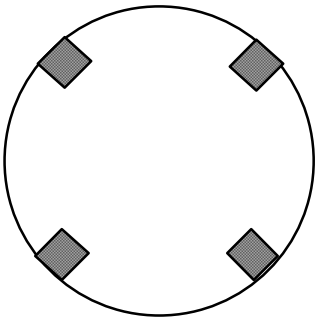
5. Why is it possible to test HIV positive and not demonstrate any symptoms of AIDS?
   Your body will begin producing antibodies against HIV shortly after you are exposed to the virus. As the viral load in your body increases, your immune system is destroyed. With a weakened immune system, susceptibility to disease increases. A person has "full blown AIDS" at the later stage of infection after immune damage has occurred.

6. Why is it suggested that a person exposed to HIV be tested once and then be tested again several months later?
   It takes a period of time before the amount of antibody against HIV rises to a detectable level. If the first test is done before this time, it will give a false negative. In a few more weeks, though, if the test is repeated it may be possible to detect the antibodies..

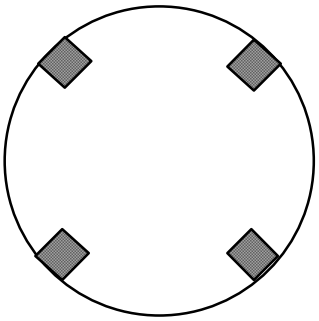
Answers to questions in the lab:

The students' sketches should look like the following:

4.

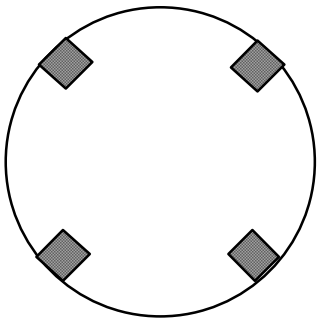


Healthy Patient Test

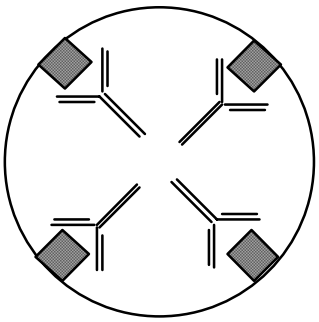


HIV Infected Patient Test

13.

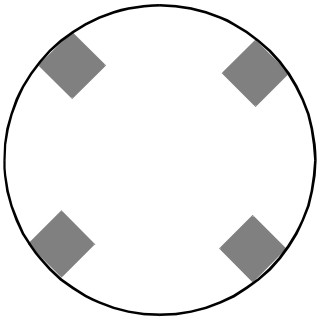


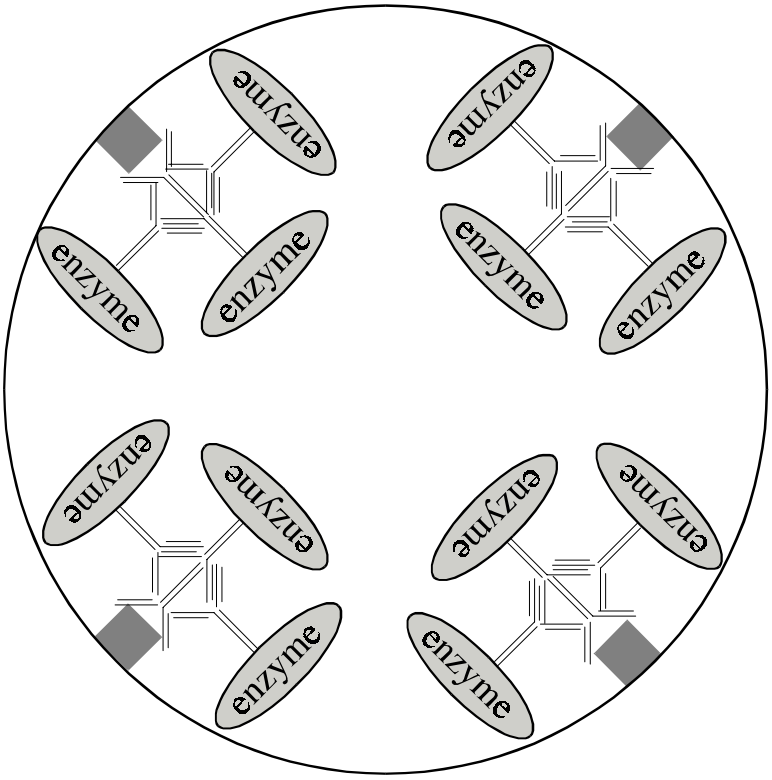
Healthy Patient Test



HIV Infected Patient Test

17.

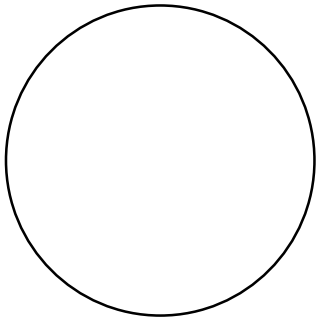


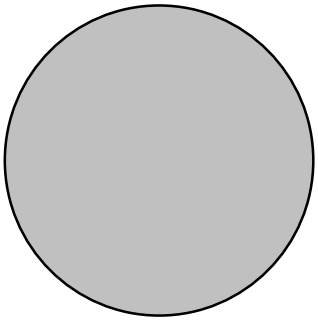


Healthy Patient Test

HIV Infected Patient Test

19.





Healthy Patient Test

HIV Infected Patient Test

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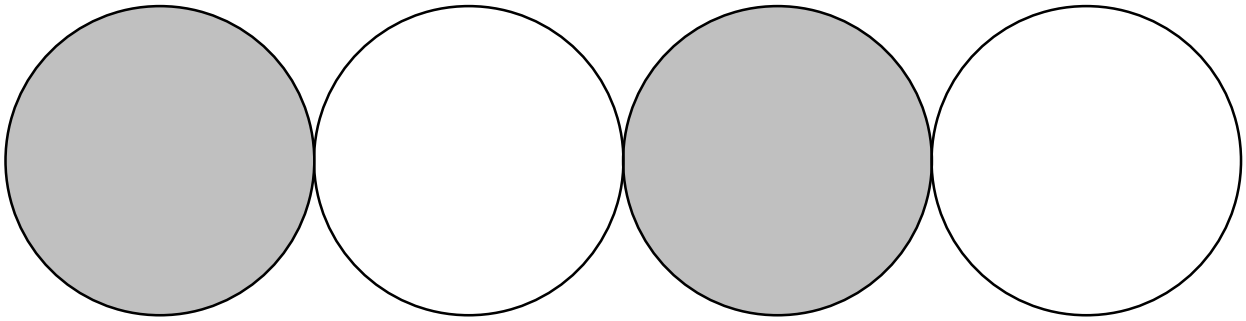


Positive Control | Negative Control | Patient #1 | Patient #2

Answers to Post-lab Questions:

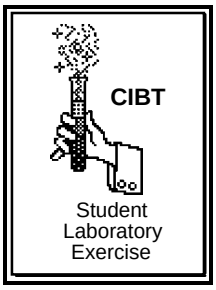
1. Which patient sample(s) are positive for HIV antibodies and which are negative? Explain how you know this.
   The blood sample from patient 1 contains antibodies against HIV and the individual is therefore infected with HIV while patient 2 is uninfected. I know this because an enzyme reaction occurred in the tube that contained the patient 1 blood sample. For enzyme to be present, human antibodies must have been present. For human antibodies to bind to the tube, antibodies against the HIV antigen must have been present in the blood sample.

2. What is the critical importance of the "positive" control reaction? In other words, what does it tell you about a negative test result if the "positive" control reaction never turns color?
   If the positive control reaction does not turn color, there is a problem with one of the reagents or the system. Since we know the positive control reaction should change color, yet didn't, a test sample that fails to change color may also be positive (but looks like a negative result). Without the positive control, that test sample would be scored as negative!

3. What is the critical importance of the "negative" control reaction? In other words, what does it tell you about a positive test result if the "negative" control reaction turns green?
   If the negative control reaction turns color, there is a problem with one of the reagents or the system. Since we know the negative control reaction should not change color, yet did, a test sample that changes color may also be negative (but looks like a positive result). Without the negative control, that test sample would be scored as positive!

4. The ELISA test indicates whether or not antibodies to HIV are present. What does this have to do with AIDS?
   If a person has produced antibodies to the HIV virus, it means that they have been exposed to and infected by the virus. Otherwise, their body would not have undergone an immune response and they would not have any antibodies against HIV in their blood.

HIV Testing



Pre-lab homework:

Name: _____ Date: _____

Consult any resource available to you to answer the following questions.

1. Give a complete citation for the reference(s) used. Your citation should be written using the correct format and punctuation for citing a reference.

2. List and describe four symptoms/ conditions commonly associated with AIDS.

   (1)

   (2)

   (3)

   (4)

3. List two ways that you can protect yourself against infection by the HIV virus.

(1)

(2)

4. What is an antibody and why are antibodies important?

5. Why is it possible to test HIV positive and not demonstrate any symptoms of AIDS?

6. Why is it suggested that a person exposed to HIV be tested once and then be tested again several months later?

Introduction

Over the past ten years, the incidence of AIDS has increased dramatically. As of January 1997, approximately five million people have died from AIDS-related diseases. Very few people in the world today have not experienced the death of a friend or loved one to the disease. Anyone who is sexually active should protect themselves and their partner from the AIDS virus by practicing “safe sex” and by getting tested for HIV infection on a regular basis. If you do not know the meaning of "safe sex", you should ask your parents, your teacher, your school nurse, or your family doctor. They can also tell you how to get tested. In this activity you will run through a simulation of the ELISA test that is used to diagnose AIDS. This test is also used to diagnose many other diseases and conditions such as Lupus and pregnancy testing.

To understand the principles underlying the HIV test, you need to know a little bit about our immune systems. Our bodies make antibodies in response to infection. An antibody binds to the infection causing agent, or antigen, and labels it for disposal much like a trash tag labels a can of trash for disposal. An antibody binds only to the antigen that caused its production. This makes an antibody an ideal laboratory chemical for recognizing a specific substance. The HIV test looks for the presence of antibodies against HIV in your blood. The complication is that blood contains many antibodies and for this test we are only interested in HIV antibodies. Since HIV antibodies bind to HIV antigen and other antibodies don't, the test is designed to see whether a blood sample contains any antibodies that bind to HIV. In this ELISA test, the test tube is coated with HIV antigens. When a blood sample is added to the tube, if antibodies against HIV are present they will bind to the HIV antigen and will remain attached to the tube. The many other antibodies in the blood sample do not bind to the HIV antigen and are washed away.

But how do we know whether any human antibodies have bound to the HIV antigen? A second antibody is used to determine whether the HIV antigen has bound human antibodies. When human antibodies are injected into an animal, the animal makes antibodies against the injected human antibodies. These animal antibodies, called secondary antibodies, are used to determine whether or not human antibodies are present. If human antibodies bound the HIV antigen, animal antibodies when added to the tube will bind to the human antibody. Following the addition of the blood sample and the secondary antibody treatment, if no HIV antibody was originally present, neither human nor animal antibodies will stick to the tube. If HIV antibodies are present in the blood sample, both human and animal antibodies will be stuck.

Animal antibodies are not any more visible than the original human antibody. To make it easy to determine or to visualize if animal antibodies are present, an enzyme is attached to the animal antibody. At the end of the test, specific substrates to the enzyme are added to the tube. If the enzyme is present, then the substrate is converted to product, and the color of the solution in the tube will change. A change in color shows that enzyme is present which shows that animal antibodies are also present. Since animal antibodies are present, human antibodies must also be present. Human antibodies would only be present if the

blood sample contained antibodies against HIV. A color change is thus a positive test result for HIV.

When a positive test result is found, the test is repeated and more rigorous tests are done. During the entire process, HIV counseling is made available to the patient to answer their questions and to help them to cope with their situation.

Procedure

You are about to do a test called an **E**nzyme-**l**inked **I**mmuno**S**orbent **A**ssay (**ELISA**). The activity is a simulation of an actual test for infection by HIV. Although the test procedure is completely accurate, the antigen present in your strips is not HIV antigen and the blood samples are not from humans. There is no danger of catching AIDS from this activity!

It is critical that each step be carried out in the order written. To help you to keep track, place a • in the space provided after you complete the step.

_____ 1. The first step in the procedure is for a small sample of blood to be drawn. A drop of the blood is dried onto a filter paper and sent to a licensed clinical laboratory for analysis. Since you are acting as a technician in this clinical lab, you will be provided with dried samples and you will carry out only the remainder of the steps.

_____ 2. Obtain a strip of four miniature test tubes from your teacher. Do not break the tubes apart!

_____ 3. Label each of the tubes on the side as follows (do not use blue ink because blue may be confused for a positive reaction after adding the substrate):

a. "P" for positive control (**known to contain** antibodies to HIV)

b. "N" for negative control (**known not to contain** antibodies to HIV)"

c. "1" for blood sample from patient #1

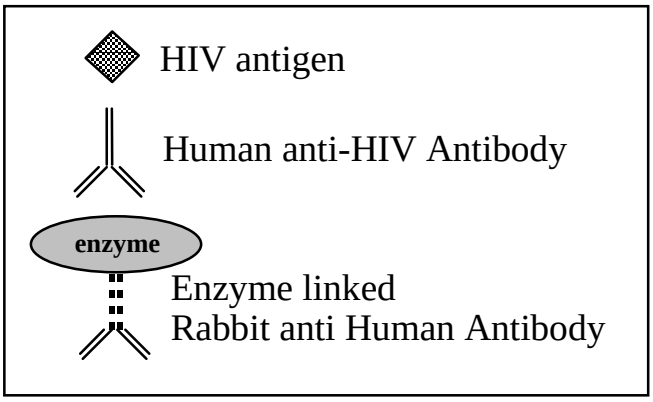
d. "2" for blood sample from patient #2

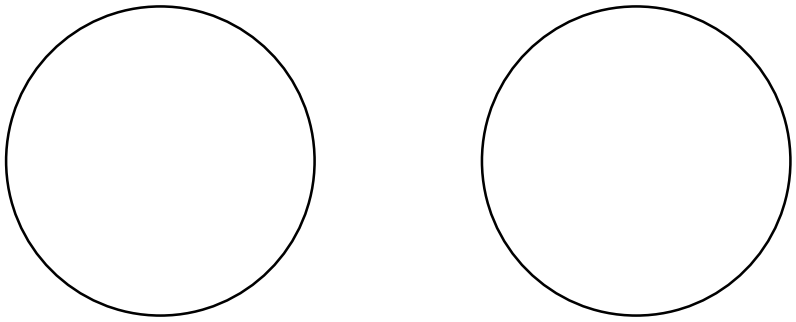
Each tube in the strip has already been coated with HIV antigen.

_____ 4. In the "mock" test tubes shown on the next page (and in similar drawings that appear later in the lab), you will be asked to draw sketches of the following **as you complete each step** in the actual lab activity:

HIV antigen
human anti-HIV antibody
enzyme-linked rabbit anti-human antibody

Fill in the diagrams of the healthy patient test and the HIV infected patient test using the symbols given in the following diagram. **Draw in only those substances that you have added up to this point in the lab.**





Healthy Patient Test HIV Infected Patient Test

_____ 5. Obtain a set of filter paper samples from your teacher. You will need one of each of the following samples:

a. Positive control (P)
b. Negative control (N)
c. Patient #1 sample (1)
d. patient #2 sample (2)

Use a different set of forceps to place the appropriate filter paper sample into the appropriately labeled tube. For example, add the positive control filter (P) to tube "P", the negative control filter (N) to tube "N", etc.

_____ 6. After all the filters have been placed, use a transfer pipette to add **2 drops** of PBS/ 3% milk to each of the four tubes. Leave the strip of tubes at room temperature for 25 minutes.

_____ 7. Use a different forceps to remove and discard the filter papers. Be sure to use a different forceps to remove each of the filter papers so that you will not contaminate one well with the contents of a different well.

_____ 8. Invert the strip of tubes over the sink and dump out the liquid. Then turn the strip upside down and tap it firmly three times onto paper towels to remove any remaining drops of liquid.

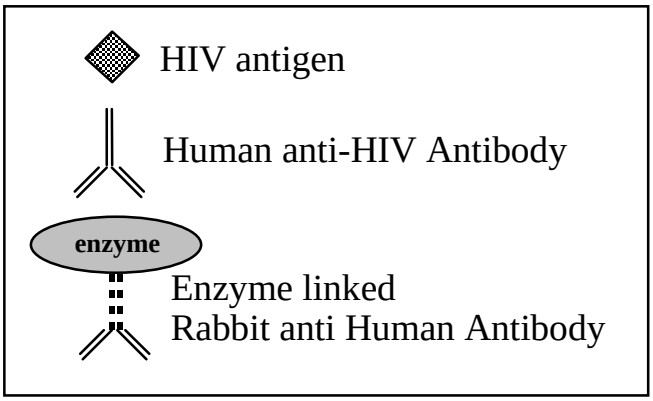
_____ 9. Using a transfer pipette, completely **fill** each tube with PBS/ Tween solution. Invert the strip over the sink and dump out the liquid. Then turn the strip upside down and once again tap it firmly three times onto paper towels to remove any remaining drops of liquid.

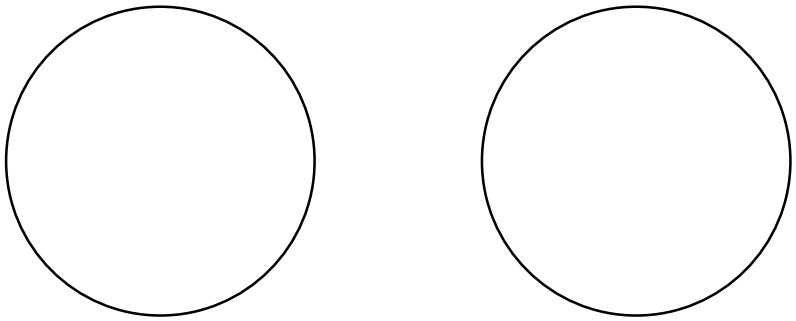
_____ 10. Fill the wells again with PBS/ Tween. Dump and tap as before.

_____ 11. Fill the wells with PBS (No Tween). Dump and tap.

_____ 12. Fill the wells with PBS (No Tween). Dump and tap.

_____ 13. In the space below, show which components will NOW be present in each tube.





Healthy Patient Test HIV Infected Patient Test

_____ 14. Add **two drops of the enzyme-linked animal antibodies (your secondary antibody)** to each tube and incubate for 5 minutes at room temperature.

_____ 15. Using the Dump and Tap technique described earlier, rinse the tubes with PBS Tween a **total of five times.**

_____ rinse 1

_____ rinse 2

_____ rinse 3

_____ rinse 4

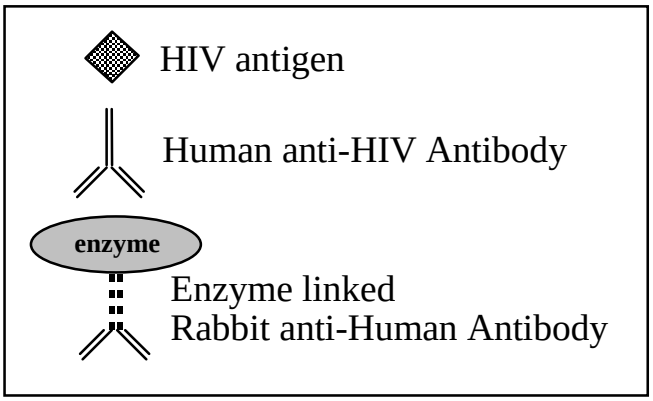
_____ rinse 5

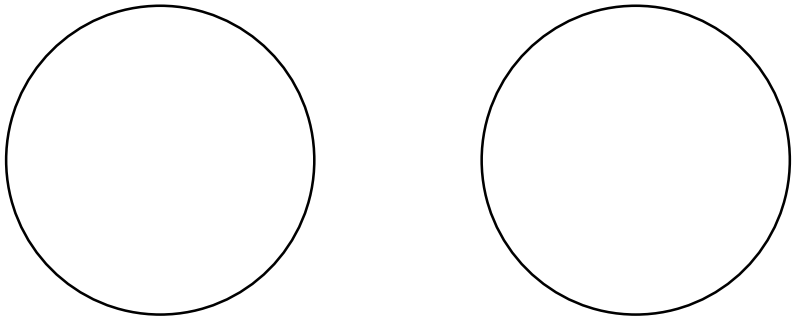
_____ 16. Using the Dump and Tap technique, rinse the tubes **twice** with PBS (No Tween).

_____ rinse 1

_____ rinse 2

_____ 17. In the space below, draw which components will NOW be present in each tube.

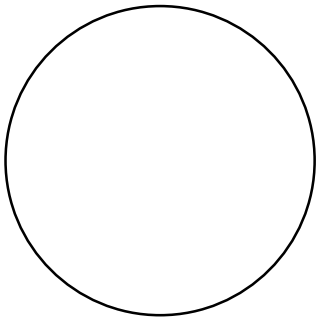




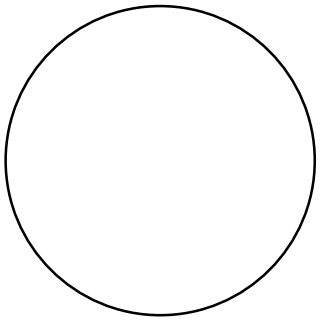
Healthy Patient Test HIV Infected Patient Test

_____ 18. Use a transfer pipette to add **4 drops of substrate solution** to each tube. Place the strip on a white background to make it easier to tell when the color begins to change from colorless to blue-green.

_____ 19. While waiting for a reaction, make a hypothesis about the expected color of each of the wells for the healthy patient and the HIV patient. Draw the expected results below.



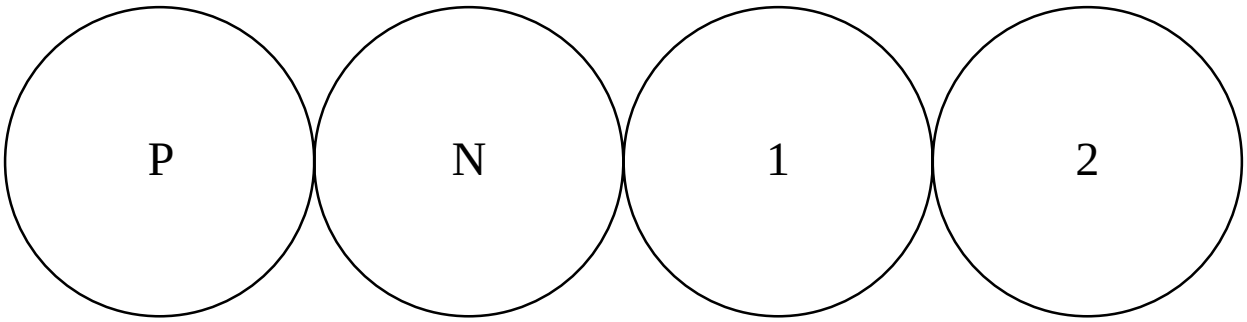
Healthy Patient Test



HIV Infected Patient Test

_____ 20. Be sure that your test tubes are on a white sheet of paper. The color change is most obvious when viewed from the side.

After a color change is visible, record your observations below.



Positive Control

Negative Control

Patient #1

Patient #2

HIV Testing

Post-lab homework:

Name: _____ Date:_____

1. Which patient sample(s) are positive for HIV antibodies and which are negative? Explain how you know this.

2. What is the critical importance of the "positive" control reaction? In other words, what does it tell you about a negative test result if the "positive" control reaction never turns color?

3. What is the critical importance of the "negative" control reaction? In other words, what does it tell you about a positive test result if the "negative" control reaction turns green?

4. The ELISA test indicates whether or not antibodies to HIV are present. What does this have to do with AIDS?

5. Using the ELISA test simulation described in this lab as a guide, design a variation that would quantify the number of antibodies found within the person being tested.

6. On your own paper, write a one page discussion covering at least one of the following topics. You may consult any reference(s) you like but be sure to include a proper citation for each reference. Where an issue has two sides, please discuss both sides and explain why you favor one side over the other.

   (a) Should everyone be required by law to have the HIV test? If yes, what should be done with the test results?

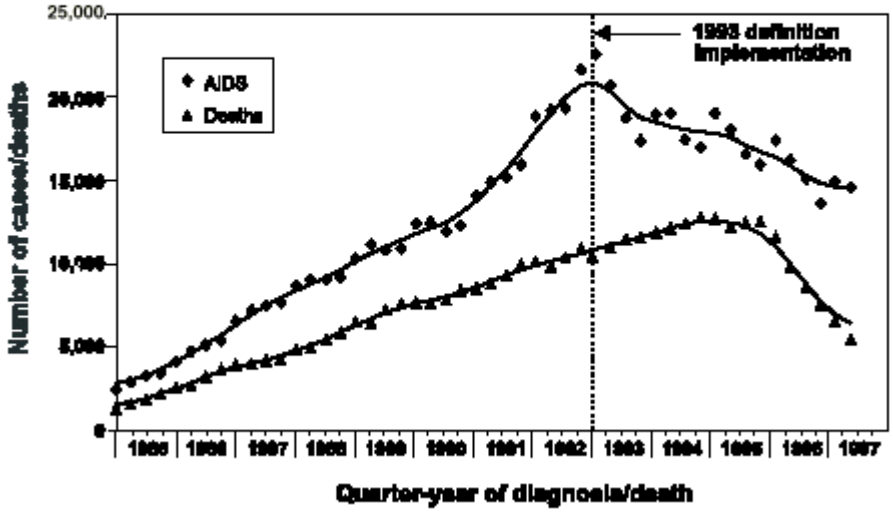
   (b) HIV test results are currently treated as confidential. Is this an important consideration? Why or why not.

   (c) Should health insurance providers be allowed to refuse benefits to people with AIDS?

   (d) To reduce the transmission of HIV, should HIV testes be administered free of charge?

7. Refer to graph below to assist you in answering the questions. (Source of the graph is from HIVAIDS Surveillance Report, Vol. 9, No. 2)

Estimated incidence of AIDS and deaths of persons with AIDS, adjusted for delays in reporting, by quarter-year of diagnosis/death, United States, January 1985 through June 1997



1. Which year represents the greatest number of deaths for AIDS?

2. Which year represents the greatest number of cases for AIDS?

3. Determine the rate for each of the following:

   a. The rate of change in the number of deaths between 1985 and 1997.

   b. The rate of change in the incident rate for AIDS between 1985 and 1997.

4. Since 1995, which rate dropped faster: the number of deaths from HIV or the incidence of HIV? What factor(s) would account for this change?

5. The number of new AIDS cases reported to CDC declined from 33,590 cases in the first six months of 1996 to 29,520 new cases reported in the first six month of 1997. Them number of new cased dropped by what percentage?