Using the ELISA Assay for Disease Detection

(procedures adapted by E. Leonhardt, CCSF with permission from WJ Grimes, L Chambers, KM Kubo, ML Narro. Transmission of a Viral Disease (AIDS) Detected by a Modified ELISA Reaction? A Laboratory Simulation. American Biology Teacher, May 1998, p. 362)

A. Objectives

Become familiar with
1. The specificity of antibody-antigen interactions;
2. Diagnostic tests using ELISA assays;
3. Cell surface interactions;
4. The importance of positive and negative controls in diagnostic tests;
5. Find out who were the two original HIV carriers.

B. Materials

Each student should have the following items before starting the laboratory:

1. Microcentrifuge tube containing "body solution"
2. Empty microcentrifuge tube
3. Disposable transfer pipet

Each group of four students should also have:

1. Plate coating buffer (contains antigens)
2. ELISA test plate
3. Positive and negative control solutions (in 1.5 ml microcentrifuge tubes)
4. Paper towels
5. Antibody solution (3 ml) in tube
6. Washing solution (in wash bottle labeled PBS)
7. Color reagent solution (3 ml) in tube labeled TMB
8. Four unused disposable transfer pipets
9. Blocking buffer

C. Before coming to lab

1. Read this handout/file; and optional Reed, R. et al., p. 95 and 99
2. Draw a protocol for this exercise. Do not forget to number the steps.

In your notebook

3. State which steps you would have to modify to obtain a negative control.

D. Theoretical background (please see also Reed, R. et al. p. 95)

The Enzyme Linked Immunosorbent Assay (ELISA) is one of several rapid diagnostic tests now available in kit form and used to identify infectious agents or body metabolites. Two commonplace examples are the rapid streptococcal identification kit and the home pregnancy test kit. The rapid streptococcal identification kit is a fixture in physicians' offices and has superceded the traditional approach of isolating *Streptococcus pyogenes* on blood agar plates. ELISAs are also commonly used for detection and/or confirmation of Lyme disease and HIV infections.

In order to understand how ELISAs work, you will need to know a little bit about immune system-pathogen interactions. I will use HIV infection as an example to clarify the roles of cell surface and antigen-antibody interactions. Proteins (gp 120) sticking out from the surface of the HIV envelope bind to cell surface receptors (CD4 and co-receptors) on target cells. Once tightly bound, the HIV envelope can fuse with the host cell membrane and enter the cell (Fig. 1).

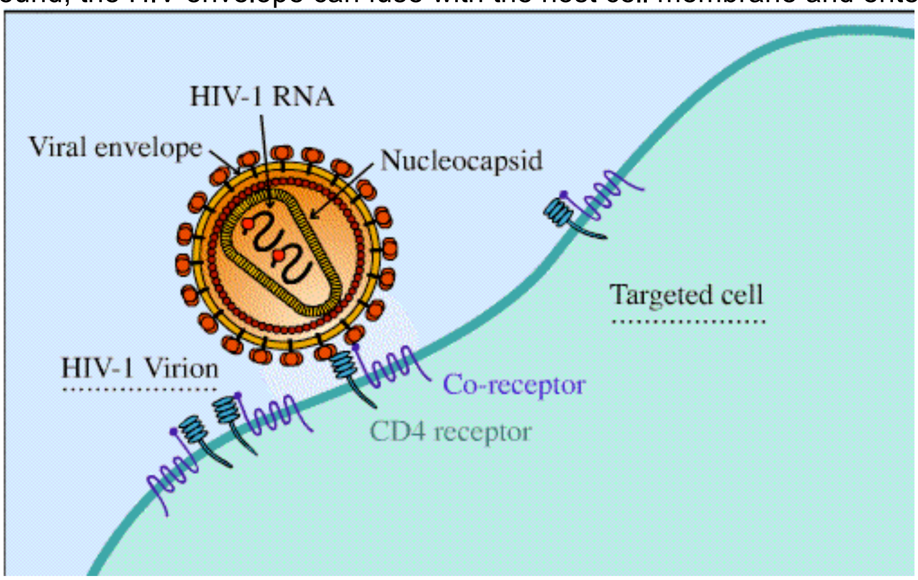

Figure 1: HIV binding to target cell (© biodidac, U of Ontario, used with permission).

The viral proteins can also act as an **antigen,** which means that in response to encountering these foreign proteins, certain cells of the immune system (B cells) produce **antibodies**. Detection of the anti-HIV antibodies to gp120 and a core protein, p24, which appear in the blood about 1 to 12 months after infection, is the most common method for identifying infected individuals. A person who is **HIV-positive** is infected, having tested positive for the presence of antibodies to the virus. The binding of antibodies to antigens tags foreign cells and molecules for destruction by the immune system, a response that helps us overcome the majority of infections. Unfortunately, HIV has several mechanisms that help it evade the immune system, one being the high rate of mutations. Since antibodies and antigens fit each other in a key-and-lock system, slight mutations in the antigen make it impossible for the existing antibodies to bind to it.

There are two main types of ELISAs: indirect, and sandwich ELISA (see Figure 2). In the indirect ELISA, the one that we will do today, antibodies indicating an infection are detected in a body fluid sample with the help of antigens attached to a solid support. In the sandwich ELISA, which is used, e.g., for pregnancy testing, the antigen is detected with the help of two antibodies.

We will do a **qualitative** ELISA, meaning that the test will allow us to distinguish between people infected with our (simulated) HIV virus and those that are not infected. ELISA results can be quantified if the intensity of color is measured using a spectrophotometer. Values are expressed as optical density (O.D.) at a particular wavelength.

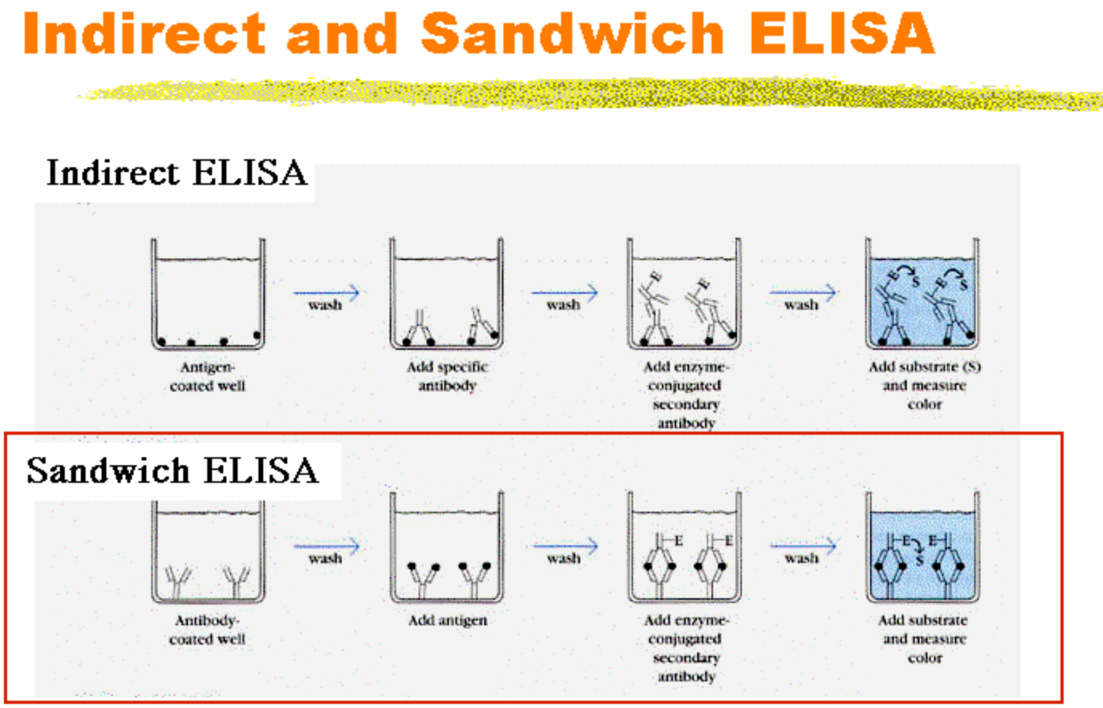

Fig. 2: Indirect and Sandwich ELISA (Source: anonymous)

In this laboratory you will obtain a microcentrifuge tube with 500 µl of (simulated) blood serum that might or might not contain antibodies to a (simulated) pathogen. First, you will exchange your blood serum with three other students. Then, we will perform an indirect ELISA (Enzyme Linked ImmunoSorbent Assay) to detect the presence of antibodies in your sample. Finally we will analyze class data to find out who the originally infected individual(s) were.

Note that we fast forward here. The normal series of event would be slower: an individual would become infected with, e.g., the HIV virus through exchange of body fluids. The virus would infect cells of the new host and within 2-3 months, the new host would produce antibodies to the virus which would then be detectable with an ELISA test.

E. Procedures during lab

Exchanging Solutions

IMPORTANT: Wait for your instructor's signal to exchange body fluids. This is to ensure that everyone has a new partner at each exchange.

1. At the start of the laboratory, you will receive a transfer pipet and a tube containing a solution that represents your "body fluids." Label the tube with your initials using the marker provided.

2. At the instructor's signal, find one other student in your class and exchange your solution with him or her. Use the transfer pipet to combine solutions into one of the sharing tubes and then divide into two equal volumes in the sharing tubes. Record the name and number of the person you first made contact with.

3. At the instructor's signal find a different student to exchange your body fluid solution with. Record the name and number of your second contact.

   Note: Be sure to choose students from all over the class, and not just in your immediate area. Wait for instructor's signal before exchanging fluids.

4. At the instructor's signal exchange your body fluid solution with one more student and record the name and number of your third contact.

ELISA Test

1. After all three contacts have been completed, you will do an ELISA test on your body fluid.

2. Form a group of four people at your lab station. Your group will run an ELISA assay on your mixtures of body fluids from each student in your group using an ELISA plate. Start the experiment by organizing all of the materials that you will need for performing this test.

3. Next, add 50 μl of plate-coating buffer to each of the 24 wells. Each student should take a turn adding this buffer.

4. Now each student should add 50 μl of their serum to the 50 μl of coating buffer in each of the three wells in column number two through five (as depicted in the diagram below).

5. Next, add 50 μl of a negative test solution into the wells in column 1 and add 50 μl of a positive test solution into the wells in column 8 (as depicted in the diagram below).

	1	2	3	4	5	6	7	8
A	Negative control	Student 1	Student 2	Student 3	Student 4			Positive control
B	Negative control	Student 1	Student 2	Student 3	Student 4			Positive control
C	Negative control	Student 1	Student 2	Student 3	Student 4			Positive control

6. After everyone has added his or her solution, incubate the plate for 30 minutes at 37 ˚C in the incubator.

7. Shake off the fluid into a nearby sink or designated container, making sure that the fluid has emptied from each well. Tap (flick) the plate upside down onto the paper towel to remove any excess liquid or bubbles.

8. Add **washing solution** to the wells to just below the rim of the well. Make sure to prevent overflow and contamination. Then, as shown by your instructor, vigorously shake off fluid. Repeat a total of three times.

9. Add 250 µl of blocking buffer to all wells and incubate for 30 minutes at room temperature.

10. Add **washing solution** to the wells as done in step 8, and repeat washing a total of three times.

11. Obtain 3 ml of anti-HIV antibody and add 100 µl of this **enzyme-linked-anti-HIV antibody** solution to each well containing samples or controls.

12. Incubate for 30 minutes at room temperature, then shake off the fluid.

13. Add **washing solution** to the wells as done in step 8, and repeat washing a total of three times.

14. Obtain 3 ml of the 3,3',5,5'-tetramethylbenzidine **(TMB**) – a colorigenic substrate. Add 100 µl of the **TMB substrate** solution to each well containing samples or controls.

15. Observe your wells for five minutes. As soon as your positive sample has turned dark blue, add 200 µl of the **stop solution** to each well containing samples or controls.

16. Each group will record the results of the tests. Be sure to record whether you are infected. You will also record your test results and list of partners on the blackboard.

17. Given the classroom data, determine the original infected carriers.

Review questions

1. Define antigen.
2. Define antibody.
3. How would you set up a positive control for this indirect ELISA?
4. What does a positive control tell you?
5. How would you set up a negative control for this indirect ELISA?
6. What does a negative control tell you?
7. Explain what is meant by a false positive test. Name one error that could result in a false positive test.
8. Explain what is meant by a false negative test. Name one error that could result in a false negative test. Suppose you finished your ELISA test. Draw a picture of what a negative well would contain at this point.
9. Suppose you finished your ELISA test. Draw a picture of what a positive well would contain at this point.
10. We first added plate coating buffer containing HIV antigens (simulated) to our wells. What is the purpose of a) the plate coating buffer and b) the HIV antigen?
11. What was the purpose of washing the plates between additions of each reagent?
12. We later added assay-blocking buffer and enzyme-conjugated anti-HIV antibodies. What was the purpose of a) the assay-blocking buffer, b) the anti-HIV antibodies and c) the conjugated enzyme?
13. What is the purpose of adding TMB?
14. What makes ELISAs specific?
15. How would you set up a sandwich ELISA to detect the presence of HIV?
16. Why is an indirect ELISA preferred over a sandwich ELISA for detecting HIV?
17. What is detected in a pregnancy ELISA?
18. After doing the lab, would you agree or disagree with the following statement: "When you have sex with someone, you are also having sex with everyone that they have previously had sex with." Explain your answer.
19. How can you protect yourself from sexually transmitted diseases?
20. How could you quantify your ELISA results?

21. What is the ELISA test intended to measure?

a) Antibody to HIV only
b) Antigen to HIV only
c) Presence of free, circulating virus in the patient
d) Antibodies directed against HLA molecules

22. What would happen if serum were omitted from the ELISA, but all other steps remained the same and were performed properly?
a) Anti-HIV antibody-conjugate would not bind and be washed away.
b) Anti-HIV antibody-conjugate would bind non-specifically to the ELISA plate.
c) The optical density values (which indicate the concentration of antibodies in the sample) would be nearly the same as for the negative control.
d) Both A and C

23. What would happen if the Anti-HIV antibody-conjugate were not washed free of the well before the substrate was added?
a) The ELISA would not develop when the substrate was added.
b) The ELISA would develop normally.
c) All wells would show uniform over-development due to unbound and excess Anti-HIV antibody-conjugate.
d) Both A and B.

F. After lab

1. Make sure all pertinent results are in your notebook. It should be clear whether you are positive or negative and if positive, from whom you have gotten the antibodies.

2. Justify your conclusions about the potential original HIV carrier(s).