Using the ELISA Assay for Disease Detection

In this laboratory you will use the ELISA (Enzyme Linked ImmunoSorbent Assay) for detecting the presence of an antigen, such as a disease-related agent, from a sample of body fluid. You will be given a solution that represents your body fluid. You will exchange body fluid with three other individuals. The ELISA test will be used to detect a simulated disease agent in the mixed body fluids. Finally, you will analyze the class data set to determine the identity of the original carrier(s).

ELISA background information
ELISA is a powerful, antibody-based technique that is widely used throughout the biological sciences and in health care for a variety of purposes. The goal of an ELISA is to test a solution for the presence of an antigen (called a Direct ELISA) or for the presence of an antibody (called an Indirect ELISA). Direct ELISAs in use today can detect bacterial toxins from Escherichia coli, Staphylococcus aureus and Vibrio cholera (which makes cholera toxin). Direct ELISAs can detect viruses such as rotavirus, hepatitis viruses, rubella virus, and measles and mumps viruses. Indirect ELISAs (that test for antibodies) have been developed for HIV, Salmonella, Mycobacterium tuberculosis, and the causative agent of Lyme disease, in addition to many other disease causing agents.

So, how do they work? A few things to keep in mind- First, antibodies bind with incredible specificity to a particular antigen. In practical terms, this means that these tests tend to be incredibly specific for the antigen/antibody in question. ELISAs are also highly sensitive assays which means that detection of a particular interaction can be easily detected.

Here are the details about a direct ELISA which is what we will be running in the lab, remember that this test detects the presence of an antigen in solution. First, an antibody specific for the antigen in question is “impregnated” or stuck to the ELISA test plate (see Fig. 1, Step 1). Antibodies are often depicted as “Y” shaped molecules. The stuck-antibodies are positioned with their “arms” up in the air, and the tail end is attached to the plate. A solution is added to the wells of the ELISA plate, and allowed to incubate. If antigen is present, it will interact with and adhere very tightly (with high affinity) to the antibody “arms” (Fig 1, Step 2). Wash steps follow. Washing will not dislodge bound antigen, but will remove any molecules that have stuck non-specifically to the plate or to the antibodies (or to antigen, even). After washing, antibody solution is added to the plate. This antibody will also bind to the antigen but from a different angle, or at a different 3-D epitope, of the antigen (Fig 1, Step 3). This binding will, again, have high specificity for only the antigen in question. Wash steps follow. The antibody added is a modified antibody. It has been manipulated to increase the sensitivity of this assay. The antibody has a covalently attached enzyme (“e” in Fig. 1) linked to the “tail” end of the antibody molecule. This enzyme is called peroxidase. Peroxidase catalyzes reactions whose product(s) are colored (Fig 1, Step 4) and can be detected in very low amounts. The substrate we use in lab turns blue when cleaved by peroxidase. Thus, blue color in a well of the ELISA plate indicates the presence of the antigen in that sample.
Figure 1- Direct ELISA

Materials:
Each student should have the following items before starting the laboratory:
- Microcentrifuge tube containing "body fluids" (BF)
- Empty microcentrifuge tube
- Disposable transfer pipet
Each group of four students should also have:
- ELISA test plate
- Positive and negative control solutions (in their respective dropper bottles)
- Paper towels
- Antibody solution (in dropper bottle)
- Washing solution (in wash bottle)
- Color reagent solution (in dropper bottle)

Procedure:

1. At the start of the laboratory, each student will need to obtain: a transfer pipet, an empty tube, and a numbered tube containing a solution that represents your "body fluids." Label the two tubes with your initials using the marker provided. Divide the solution in your sharing tube equally with the second tube (the empty, non-sharing tube), and set the non-sharing tube in the provided rack. Use the sharing tube for the instructions below. The non-sharing tube will be stored for later analysis.

2. **WHEN YOUR INSTRUCTOR TELLS YOU TO BEGIN**, find one other student in your class and exchange your solution from your sharing tube with him or her. Use a transfer pipet to combine solutions into one of the sharing tubes. Mix the combined solution by closing lid, and flicking tube with your finger. Open tube and then divide the mixture into two equal volumes back into the sharing tubes. **Record the name of the person you first made contact with, and their body-fluid number. Table provided below for this data!**

3. At the instructor's NEXT signal, find a different student with whom you will exchange your sharing solution. Repeat instructions as above. **Record the name 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 sharing solution solution with one more student, as above, and record the name of your third contact.
ELISA Test

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

2. Form a group (usually three or 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.

3. Using your transfer pipet, add three (3) drops of your sharing tube fluid into each of three wells. Record which wells contain your fluid to avoid confusing your wells with another student's. Understand that you are running your sample in “triplicate.”

4. Each group will also do positive and one negative controls in their ELISA plate. Using the dropper in the bottle, add two (2) drops of a positive control solution into three wells and two (2) drops of a negative control solution into a different set of three wells. Each group is running positive and negative controls in triplicate.

5. After everyone has added their solution, leave the plate on the lab table undisturbed for five minutes.

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

7. Gently add washing solution to the wells as shown by your instructor, and shake off fluid. Repeat a total of three times.

8. Add two (2) drops of the antibody solution to each utilized well.

9. Allow five minutes incubation time on the lab table and then shake off the fluid.

10. Add washing solution to the wells as done in step 7, and repeat washing a total of three times.

11. Add two (2) drops of the color reagent solution to each well.

12. Record your observations after five minutes.

13. Each group will record the results of the tests. Be sure to record whether you are infected. Positive ELISA results (and partner-sharing information) will be shared with the class, on the board (wait for instructor’s direction).

14. Given the classroom data, propose a list of suspected, original infected carrier(s). After identifying potential sources of the disease, you will next confirm/disprove your analysis by performing another ELISA assay on the non-shared samples.

15. Record this data and share it with the class. (Follow instructions as above).

TABLE for Recording “SWAPPING” DATA

<table>
<thead>
<tr>
<th>1st Partner #</th>
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<tbody>
<tr>
<td>2nd Partner #</td>
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<tr>
<td>3rd Partner #</td>
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ELISA- Laboratory Questions
Answers may be (but are not required to be) submitted as group work.

1. We ran both positive and negative controls in this experiment.
   A. What ingredients were in the positive control wells?
   B. What unique, and important piece of information did you learn from the results of the positive control in this experiment? (Hint: it is NOT that the procedural steps of washing worked, or that the peroxidase worked)
   C. What ingredients were in the negative control wells (and/or what was lacking from these wells)?
   D. What specifically did you learn from the results of the negative control in this experiment?

2. What was the purpose of washing the plates between the addition of each reagent?

3. A. In your own words, explain what is meant by a false positive test result.
   B. Name two different errors/problems that would result in a false positive test result for this ELISA.

4. A. In your own words, explain what is meant by a false negative test result.
   B. Name two different errors/problems that would result in a false negative test result for this ELISA.

5. Direct ELISAs are sometimes referred to as “sandwich ELISAs”. Why? What is sandwiched? By what?
6. ELISA’s are both specific and sensitive.
   A. Explain why antibody specificity is crucial to the functioning of the ELISA test.

   B. What component(s) of the ELISA provide sensitivity?

7. Direct ELISA is frequently used to test for the presence of bacterial toxins in the body fluids. Conversely, Indirect ELISA is more likely to be employed to test for HIV infection. Indirect ELISA can also be used (albeit infrequently) to test for infection by *Chlamydia trachomatis*, the causative agent of the sexually transmitted disease, chlamydia. Why is Indirect ELISA a better test for both HIV infection and Chlamydia than a Direct ELISA? (Hint: *Chlamydia trachomatis* is an obligate intracellular parasite).