Purpose
To use antibodies found in simulated patient serum to detect the presence of HIV infection through an ELISA protocol.

Big Idea
Antibodies are Y-shaped proteins used by the immune system to identify and help remove foreign objects like bacteria and viruses. Their specific shape helps them to recognize many different antigens (the unique parts of a substance that causes an immune response).

An ELISA (enzyme-linked immunosorbent assay) uses designed antibodies to detect if a particular substance, such as a viral antigen, hormone, or another specific antibody, is present in a sample.

In this lab, you will use an ELISA protocol that will simulate the difference between HIV positive and HIV negative blood serum samples. First, you will add the primary antigen (p24), which will bind to the surface of the 96 well plate. p24 is a protein found on the HIV virus. Second, you will add the “patient’s samples” to each coated well, followed by a secondary antibody (anti-HIV enzyme-linked antibody) and substrate to visualize a signal. By comparing your “patient” results to positive and negative controls, you will be able to determine if the samples were positive or negative for HIV antibodies.

As a hospital medical technician, you have been given four “blood serum samples” from patients who suspect they are at risk for HIV:

A. Female with partner who is HIV-positive
B. Nurse who worked in Sub-Saharan Africa for 3 months
C. Sexually-active female
D. 18-year-old male who kissed HIV-positive partner

Copy Table 1 in your lab notebook and predict whether you think each patient will test positive or negative for HIV antibodies.

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>HIV negative</th>
<th>HIV positive</th>
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<tbody>
<tr>
<td>A</td>
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<td>D</td>
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</table>

Table 1 Class Experimental Prediction

Your group will be testing two of the four patient samples with positive and negative controls. To ensure that your results are accurate, all your samples will be run in triplicate.
Materials per station

- goggles/gloves
- Eppendorf rack
- Paper towels
- 96 well plate
- micropipet (P200)/tips
- timer
- tip waste container
- transfer pipet

- permanent marker
- 750 µl p24 antigen (green)
- 175 µl positive control (pink)
- 175 µl negative control (purple)
- 175 µl each patient sample (clear)
- 750 µl secondary anti-HIV antibodies (yellow)
- 750 µl substrate (brown)

Procedures

Part 1: Plan for Loading the ELISA Plate

1. Each group will have 2 of the 4 patient samples. You will run your samples in triplicate (3 wells per control and patient sample. That’s 12 wells total.)
2. In your notebook mark which samples you will be testing.
3. Paste a copy of Figure 2 into your lab notebook and record the location of your controls and patient samples. Run the samples near each other so that the plate can be used again by the next class.

Part 2: Perform ELISA

4. Add 50 µl of HIV p24 antigen (green tube) to each of the 12 wells. Discard pipet tip.
5. Incubate for 5 minutes at room temperature. This allows for the antigen to bind to the plastic wells.
6. Remove the unbound p24 antigen.
   a. Turn the 96-well plate upside down on a stack of paper towels.
   b. Bang it forcefully several times to remove any unbound antigen.
   c. Discard the top layers of paper towels (see Figure 3).
7. **Wash Step.**
   a. Use a transfer pipet to fill each well with wash buffer. Be careful not to touch the sides of the wells with the tip. PLEASE REUSE TRANSFER PIPET FOR FUTURE WASHES.
   b. Remove the buffer by turning the 96-well plate upside down on a stack of paper towels and banging it forcefully several times.
   c. Discard the top layer of paper towels and keep the transfer pipet for future wash steps.

8. Add 50 µl of each patient sample to each of the wells you designated above on your 96-well template. Be sure to use a fresh pipet tip after pipetting a sample in triplicate.
   a. 3 wells (+) control (pink tube)
   b. 3 wells (-) control (purple tube)
   c. 3 wells patient ______ sample (clear tube)
   d. 3 wells patient ______ sample (clear tube)

9. Incubate 5 minutes at room temperature. This allows for the HIV antibodies, if present in the patient sample, to bind to p24 antigens.

10. After the 5 minutes, remove the sample using the same technique you used to remove the unbound antigen.
    a. Turn the 96-well plate upside down on a stack of paper towels.
    b. Bang it forcefully several times to remove any unbound antigen.
    c. Discard the top layers of paper towels (see Figure 3).

11. Use the same transfer pipet to wash the wells as you did in the last wash step.

12. Add 50 µl of **secondary antibodies** (yellow tube) to all 12 wells. Change the pipet tip after each triplicate. The secondary antibody is an anti-HIV antibody that is bound to an enzyme. These enzyme-bound antibodies are called “conjugated”. This enzyme interacts with the substrate we will add next, allowing us to visualize whether the anti-HIV antibodies have bound.

13. Incubate for 5 minutes at room temperature.

14. Remove the secondary antibodies.
    a. Turn the 96-well plate upside down on a stack of paper towels.
    b. Bang it forcefully several times to remove any unbound antigen.
    c. Discard the top layers of paper towels (see Figure 3).

15. Repeat the wash step 3 times to remove all unbound antibodies.

16. Add 50 µl of peroxidase substrate (brown tube) to each well. Be careful not to touch the sides of the well with the tip.

17. Incubate for 5 minutes then record your results.

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**Clean Up**
- Dispose of used tips, microtubes, and used paper towels.
- Keep waste containers and transfer pipets.
- Wash your hands. Clean up your lab station.
**Figure 5 Positive ELISA Schematic.** The p24 antigens are attached to the surface of the well. Next, the HIV antibodies from the patient serum attach to the antigen. Enzyme-linked anti-HIV antibodies then bind to the patient’s antibodies. Once the substrate is added, the HRP enzyme will convert the non-colored substrate to a colored product.

**Figure 6 Negative ELISA Schematic.** The p24 antigens are attached to the surface of the well. Next, HIV-negative sample is added. Because there are no HIV antibodies from the patient’s sample, the enzyme-linked anti-HIV antibodies cannot bind and are washed out. Without the HRP enzyme, the substrate is not converted into the visible blue product.