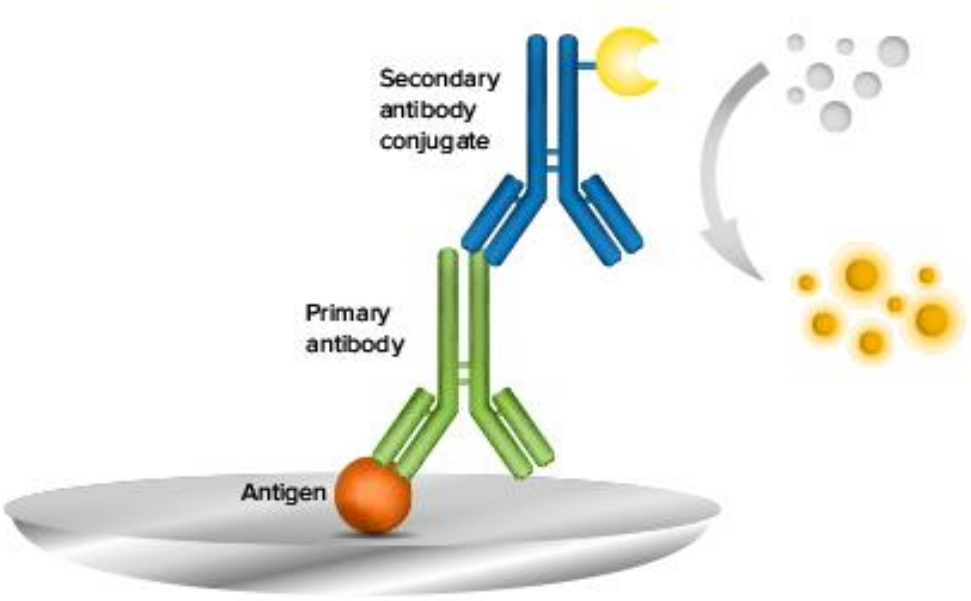
Practical Session 2 – ELISA

Learning Outcomes

- **To understand how ELISA (Enzyme-linked immunosorbent assay) can be used to determined antigen and / or antibody concentrations in blood samples:**
  - **ELISA Protocol II (Antigen Detection)**
  - **ELISA Protocol III (Antibody Test)**
- **To qualitatively determine the ELISA result on an unknown sample**



- **For protocol II (Antigen detection) this method will be determine if the antigen in the above diagram is present or not.**
- **For protocol III (antibody detection) the method will determine if the primary antibody in the above diagram is present or not.**
- **If the patient sample is positive then the secondary antibody, that is conjugated with an enzyme, will from part of the link and act on a substrate – this will cause a colour change.**

ELISA Protocol II (Antigen Detection)

Reagents (protocol II – HIV antigen test)

Tube contents	Tube colour	To simulate:
Purified antigen or Wash buffer	Yellow	2 x patient Samples (?containing HIV antigens)
Primary antibody	Green	Anti-p24 capsid protein antibody from antibody mouse
Secondary antibody	Orange	Anti-mouse immunoglobulin antibody antibody - conjugated to HRP
Positive control	Violet	Heat-inactivated viral antigen (p24 protein)
Negative control	Blue	HIV negative human serum
Enzyme substrate	Brown	

Additional consumables

- 12-well microplate strips
- 50 µl fixed-volume micropipette / 20–200 µl adjustable micropipette
- Yellow tips
- Disposable plastic transfer pipet
- 20 - 30 mls wash buffer in beaker (Phosphate buffered saline)
- Large stack of paper towels (with 0.05% Tween 20)
- Black marking pen

Before starting this practical, please ensure all reagents have been allowed to come to room temperature.

Protocol II introduction: Detecting HIV Capsid Protein

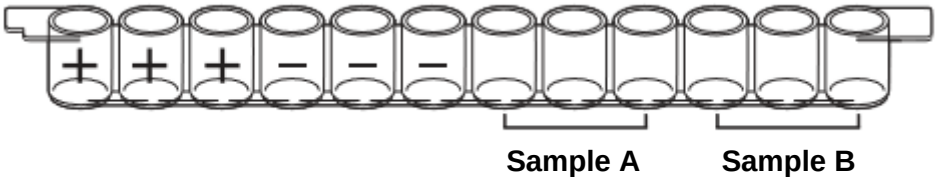
You are about to perform an ELISA (enzyme-linked immunosorbent assay). This ELISA protocol relies on antibodies to detect the presence of antigens in liquid samples. Because they are antibody-based, ELISAs are called immunoassays. ELISAs can rapidly detect minute amounts of disease agents in samples such as body fluids (before the body has had a chance to mount an immune response).

Task

You are required to test, using ELISA method, a set of unknown samples (sample A and Sample B) to determine if HIV Capsid Protein (antigen) is detected. Ensure positive and negative controls are included and that ALL samples are run in triplicate.

Method

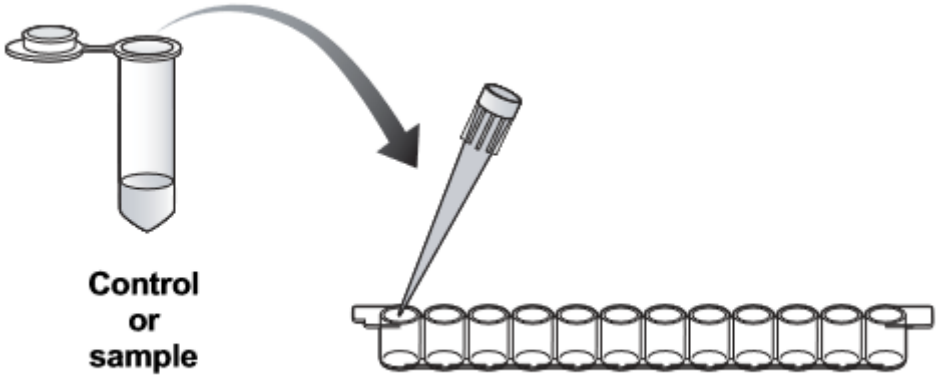
1. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "–" for the negative controls. Label the remaining wells with your samples:



2. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.

3. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.

4. Transfer 50 µl of each of your unknown samples into the appropriately labelled three wells, using a fresh pipet tip for each sample.



5. Wait 5 minutes while the proteins in the samples bind to the plastic wells.

Wash step

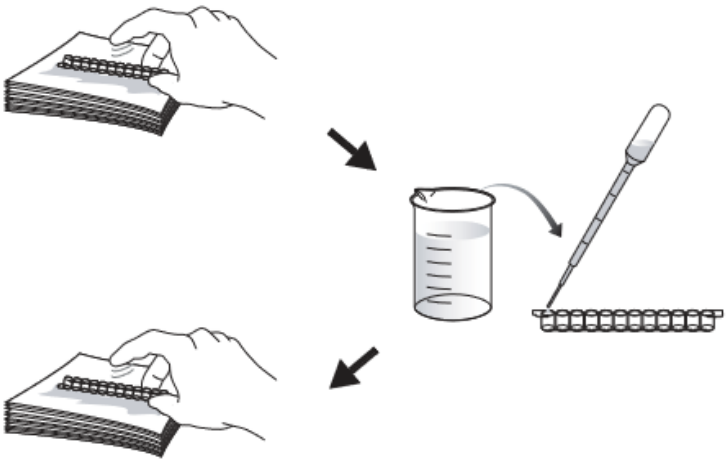
6. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid splashing samples back into wells.

7. Discard the top paper towel.

8. Use your transfer pipet to fill each well with wash buffer, taking care not to spill over into neighbouring wells. Note: the same transfer pipet is used for all washing steps.

9. Tip the microplate strip upside down onto the paper towels and tap.

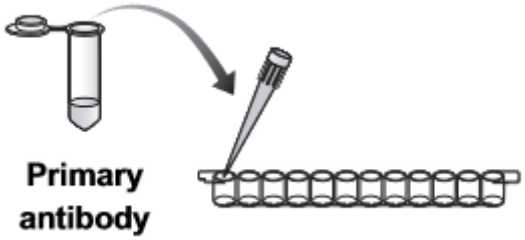
10. Discard the top 2–3 paper towels.



11. Repeat wash step above

Primary antibody binding step

12. Use a fresh pipet tip to transfer 50 µl of primary antibody (PA) into all 12 wells of the microplate strip.

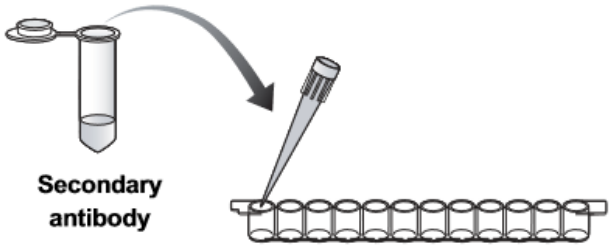


13. Wait 5 minutes for the antibodies to bind to their targets.

14. Wash the unbound primary antibody out of the wells by repeating x 2 wash steps.

Secondary antibody binding step

15. Use a fresh pipet tip to transfer 50 µl of secondary antibody (SA) into all 12 wells of the microplate strip

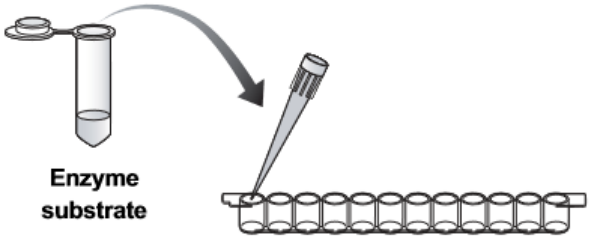


16. Wait 5 minutes for the antibodies to bind to their targets.

17. Wash the unbound secondary antibody out of the wells by repeating x 3 wash steps.

Enzyme substrate (SUB) step

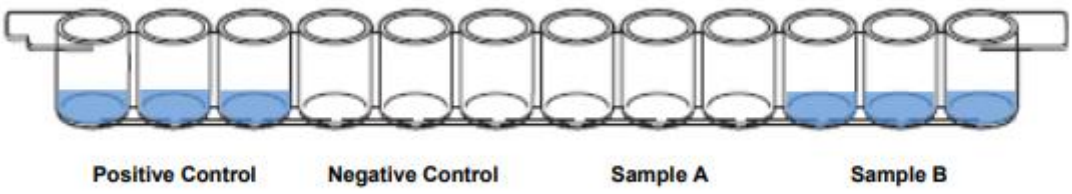
18. Use a fresh pipet tip to transfer 50 µl of enzyme substrate (SUB) into all 12 wells of the microplate strip.



19. Wait 5 minutes. Observe and record the results.

Results

Record your results below (colour = positive result)



ELISA Protocol III (Antibody Test)

Reagents (protocol III – HIV antibody test)

Tube contents	Tube colour	To simulate:
Primary antibody or Wash buffer	Yellow	2 x patient Samples (?containing HIV antibodies)
Purified antigen	Green	Purified HIV proteins
Secondary antibody	Orange	Anti-human immunoglobulin antibodies conjugated to HRP
Positive control	Violet	Serum from an HIV negative patient spiked with HIV antibodies
Negative control	Blue	Serum from an HIV negative patient
Enzyme substrate	Brown	

Additional consumables

- 12-well microplate strips
- 50 µl fixed-volume micropipette / 20–200 µl adjustable micropipette
- Yellow tips
- Disposable plastic transfer pipet
- 20 - 30 mls wash buffer in beaker (Phosphate buffered saline)
- Large stack of paper towels (with 0.05% Tween 20)
- Black marking pen

Before starting this practical, please ensure all reagents have been allowed to come to room temperature.

Protocol III introduction: Detecting HIV Antibody

You are about to perform an ELISA or enzyme-linked immunosorbent assay, this protocol can detect antibodies in your blood to determine if you have been exposed to a disease.

This protocol simulates a diagnostic blood test for the detection of serum antibodies. With this protocol, students perform an ELISA to detect circulating antibodies in the blood as an indication of exposure to a disease-causing agent. Each student is provided with a simulated serum sample and asked to assay the sample for the presence of antibodies.

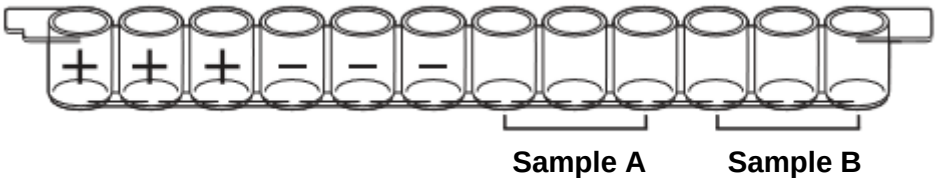
This technique is useful for detection and diagnosis “post-infection” where the antigen itself is undetectable in the body. Once the body has mounted an immune response, antibodies are present in the blood serum and can be detected.

Task

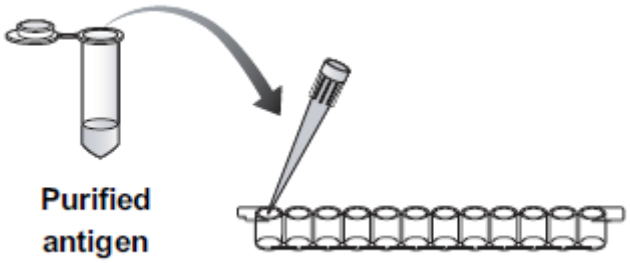
You are required to test, using ELISA method, a set of unknown samples (sample A and Sample B) to determine if HIV immunoglobulin antibodies are detectable. Ensure positive and negative controls are included and that ALL samples are run in triplicate.

Method

1. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "–" for the negative controls. Label the remaining wells with your samples:



2. Use a fresh pipet tip to transfer 50 µl of purified antigen (AG) into all 12 wells of the microplate strip



3. Wait 5 minutes for the antigen to bind to the plastic wells.

Wash step

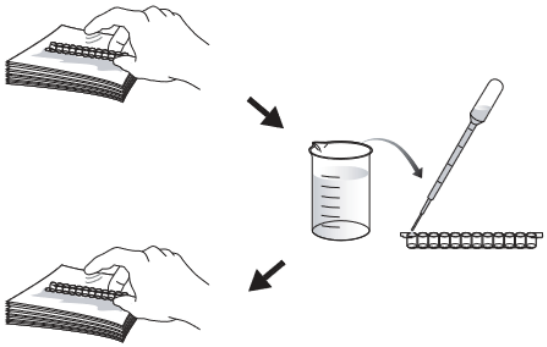
4. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid splashing samples back into wells.

5. Discard the top paper towel.

6. Use your transfer pipet to fill each well with wash buffer, taking care not to spill over into neighbouring wells. Note: the same transfer pipet is used for all washing steps.

7. Tip the microplate strip upside down onto the paper towels and tap.

8. Discard the top 2–3 paper towels.



9. Repeat wash step above

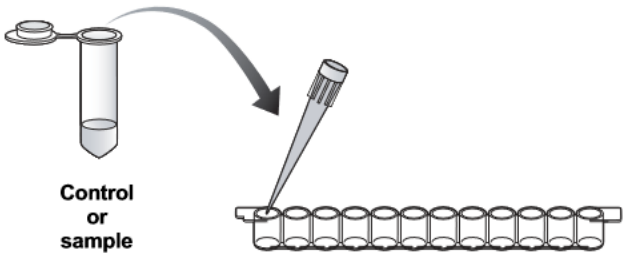
Adding samples / controls step (primary antibody)

10. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.

11. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.

12. Transfer 50 µl of each of your unknown samples into the appropriately labelled three wells, using a fresh pipet tip for each sample.

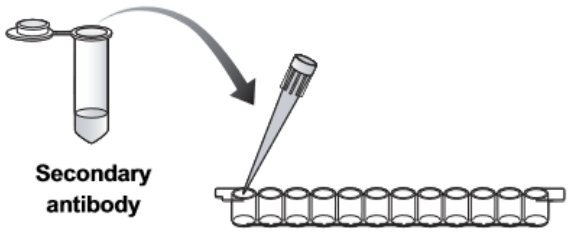
13. Wait 5 minutes for the antibodies to bind to their targets.



14. Wash the unbound primary antibody out of the wells by repeating x 2 wash steps.

Secondary antibody binding step

15. Use a fresh pipet tip to transfer 50 µl of secondary antibody (SA) into all 12 wells of the microplate strip

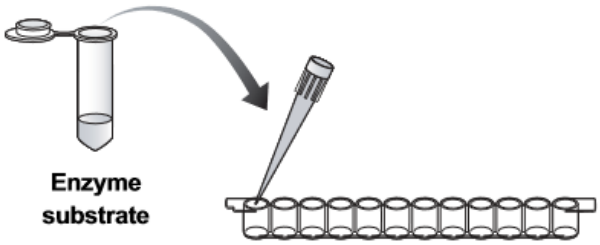


16. Wait 5 minutes for the antibodies to bind to their targets.

17. Wash the unbound secondary antibody out of the wells by repeating x 3 wash steps.

Enzyme substrate (SUB) step

18. Use a fresh pipet tip to transfer 50 µl of enzyme substrate (SUB) into all 12 wells of the microplate strip.



19. Wait 5 minutes. Observe and record the results.

Results

Record your results below: