

Lab 15 - ELISA Antibody Test

Purpose: The purpose of this laboratory experiment was to understand and implement the Enzyme-Linked Immunosorbent Assay (ELISA) as a diagnostic tool to detect the presence of specific antibodies in a given sample. ELISA is a widely used technique in immunology, providing a sensitive and specific method for detecting and quantifying antigens or antibodies in various biological samples.

Procedures:

Laboratory Quick Guide

ELISA Antibody Test

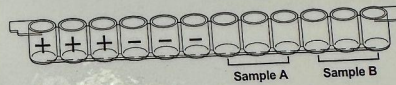
Student Workstation Checklist

One workstation serves 4 students.

Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.25 ml)	4	<input type="checkbox"/>
Violet tube (+)	Positive control (0.5 ml)	1	<input type="checkbox"/>
Blue tube (-)	Negative control (0.5 ml)	1	<input type="checkbox"/>
Green tube (AG)	Purified antigen (1.5 ml)	1	<input type="checkbox"/>
Orange tube (SA)	Secondary antibody (1.5 ml)	1	<input type="checkbox"/>
Brown tube (SUB)	Enzyme substrate (1.5 ml)	1	<input type="checkbox"/>
12-well microplate strips		2	<input type="checkbox"/>
50 μ l fixed-volume micropipet or 20–200 μ l adjustable micropipet		1	<input type="checkbox"/>
Yellow tips		10–20	<input type="checkbox"/>
Disposable plastic transfer pipet		1	<input type="checkbox"/>
70–80 ml wash buffer in beaker	Phosphate buffered saline with 0.05% Tween 20	2	<input type="checkbox"/>
Large stack of paper towels		1	<input type="checkbox"/>
Black marking pen			<input type="checkbox"/>

1. Label the yellow tubes (if necessary) to identify the samples being tested.

2. 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 to identify the samples being tested (3 wells each).

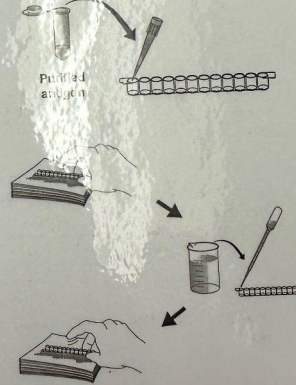


3. Use a fresh pipet tip to transfer 50 μ l of purified antigen (AG) into each of the 12 wells of the microplate strip.

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

5. WASH:

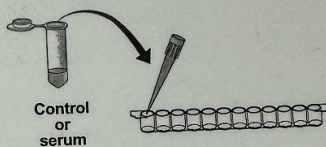
- Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid splashing sample back into wells.
- Discard the top paper towel.
- Use your transfer pipet to fill each well with wash buffer, taking care not to spill over into neighboring wells. Note: the same transfer pipet is used for all washing steps.



PROTOCOL III
ELISA Antibody Test

- d. Tip the microplate strip upside down onto the paper towels and tap.
- e. Discard the top 2–3 paper towels.
6. Repeat wash step 5.
7. Use a fresh pipet tip to transfer 50 μ l of the positive control (+) into the three "+" wells.
8. Use a fresh pipet tip to transfer 50 μ l of the negative control (–) into the three "–" wells.
9. Transfer 50 μ l of each of your team's serum samples into each of the appropriately initialed three wells, using a fresh pipet tip for each serum sample.
10. Wait 5 minutes for the antibodies to bind to their targets.

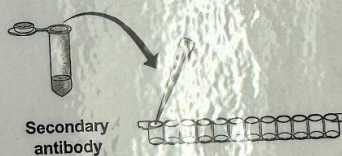
WASH



11. Wash the unbound primary antibody out of the wells by repeating all of wash step 5 **two** times.

WASH 2x

12. Use a fresh pipet tip to transfer 50 μ l of secondary antibody (SA) into each of the 12 wells of the microplate strip.

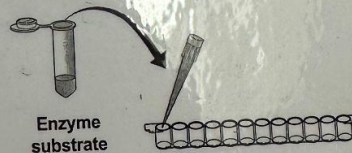


13. Wait 5 minutes for the antibodies to bind to their targets.

14. Wash the unbound secondary antibody out of the wells by repeating wash step 5 **three** times.

WASH 3x

15. Use a fresh pipet tip to transfer 50 μ l of enzyme substrate (SUB) into each of the 12 wells of the microplate strip.



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



Results:



Discussion: The ELISA test's sensitivity and specificity are crucial in obtaining accurate results. False positives or negatives may occur due to factors like cross-reactivity or inadequate washing steps. The chosen antigen and antibody pair must have high affinity to ensure a reliable outcome.

Conclusion: In conclusion, this laboratory experiment successfully demonstrated the principles and applications of the Enzyme-Linked Immunosorbent Assay (ELISA) for detecting antibodies in serum samples. The specificity and sensitivity of ELISA make it an invaluable tool in medical diagnostics and research. Understanding the ELISA method is crucial for obtaining reliable results, and this experiment contributes to the foundational knowledge required for utilizing ELISA in various immunological studies and clinical settings.