

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 sample. ELISA is a used technique in immunology, which provides a sensitive and specific method for detecting and quantifying antigens or antibodies in many biological samples.

Procedures

Laboratory Quick Guide
ELISA Antibody Test
Student Workstation Checklist
 One workstation serves 4 students.

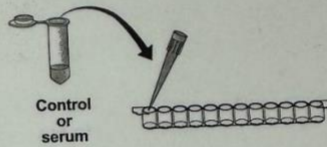
Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.25 ml)	4	<input type="checkbox"/>
Violet tube (+)	Positive control (0.5 ml)	1	<input type="checkbox"/>
Blue tube (-)	Negative control (0.5 ml)	1	<input type="checkbox"/>
Green tube (AG)	Purified antigen (1.5 ml)	1	<input type="checkbox"/>
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	1	<input type="checkbox"/>
Large stack of paper towels		2	<input type="checkbox"/>
Black marking pen		1	<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:
 - a. 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.
 - b. Discard the top paper towel.
 - c. 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

- 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.

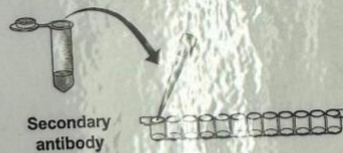
WASH



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.

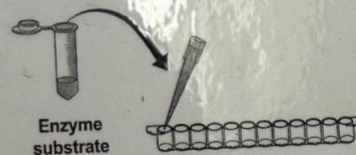


13. Wait 5 minutes for the antibodies to bind to their targets.

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

WASH 3x

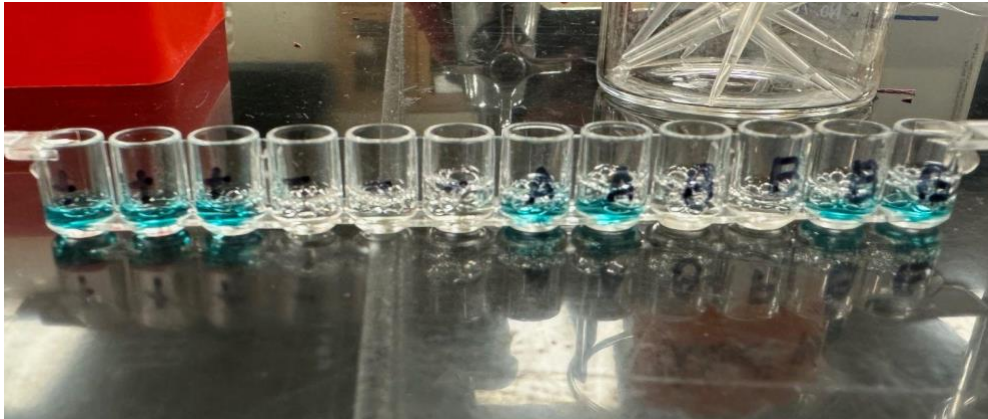
15. Use a fresh pipet tip to transfer 50 μ l of enzyme substrate (SUB) into each of the 12 wells of the microplate strip.



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



Results



Discussion

I think this lab experiment was pretty cool. I don't think I'll ever have to do this in my life, but it was cool seeing how it was done and learning about the purpose of it. It took many steps and washes so I'm wondering if there's a faster way of doing it out there in the actual lab tech world so they can get results faster.

Conclusion

In conclusion, this laboratory experiment was successful in the demonstration of principles and applications of the Enzyme-Linked Immunosorbent Assay (ELISA) test used for detecting antibodies in serum samples. The specificity of ELISA makes it an very important tool in medical diagnostics. Understanding the ELISA method is necessary for obtaining accurate results. This experiment contributes to the foundational knowledge required for utilizing ELISA in various immunological studies and clinical settings.