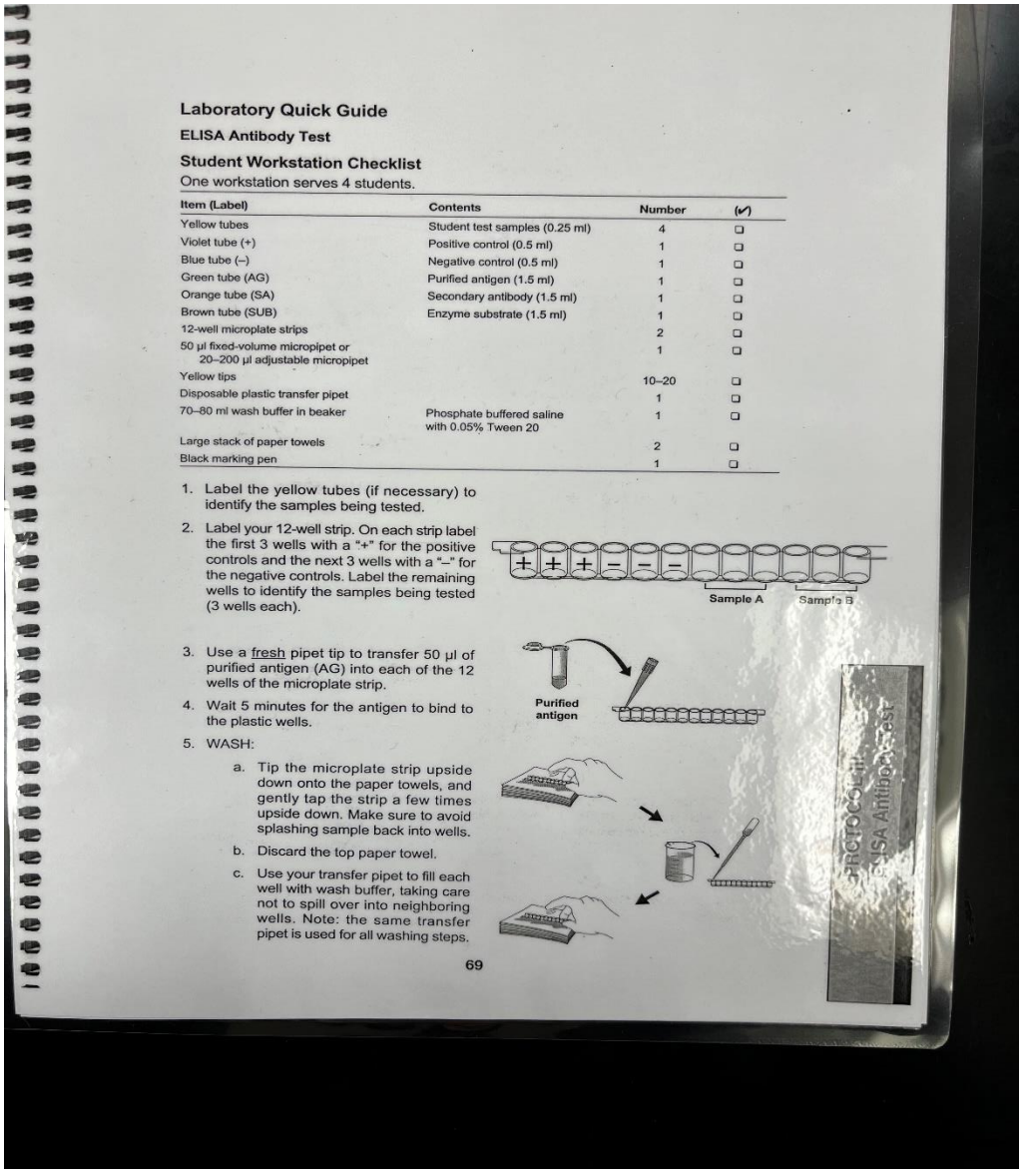
Cynthia Ramirez
December 2, 2023

Laboratory 15 ELISA Antibody Test

Purpose:

The purpose of the ELISA Antibody Test is to detect and measure antibodies against bacteria, it could be viral or fungal infections. The antibodies are proteins that our bodies produce that help us fight against antigens.

Procedure:



Laboratory Quick Guide

ELISA Antibody Test

Student Workstation Checklist

One workstation serves 4 students.

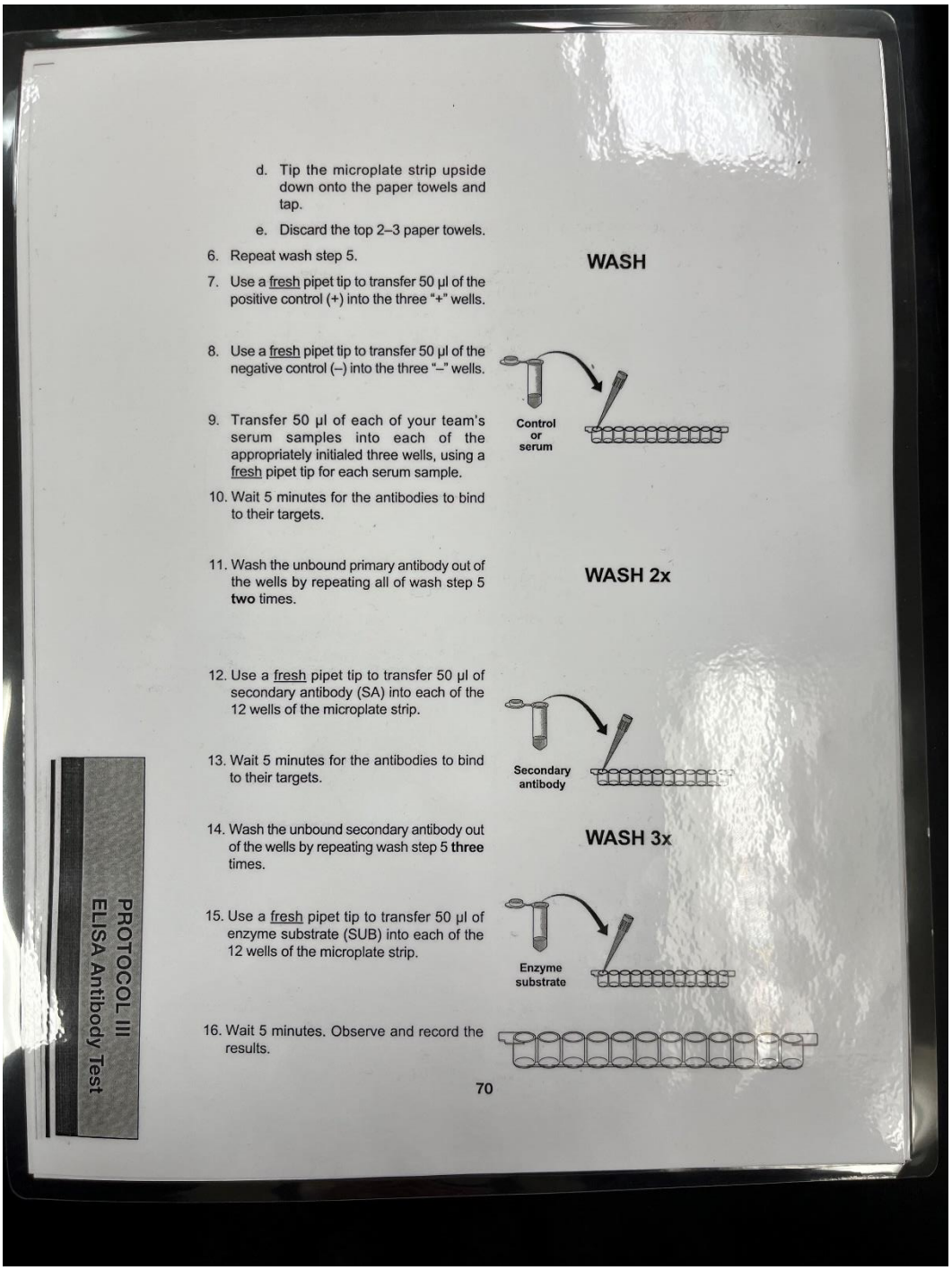
Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.25 ml)	4	❑
Violet tube (+)	Positive control (0.5 ml)	1	❑
Blue tube (–)	Negative control (0.5 ml)	1	❑
Green tube (AG)	Purified antigen (1.5 ml)	1	❑
Orange tube (SA)	Secondary antibody (1.5 ml)	1	❑
Brown tube (SUB)	Enzyme substrate (1.5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

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 µl 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.

69



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.

WASH

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

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

9. Transfer 50 µl 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.

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 µl 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 µl of enzyme substrate (SUB) into each of the 12 wells of the microplate strip.

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

PROTOCOL III
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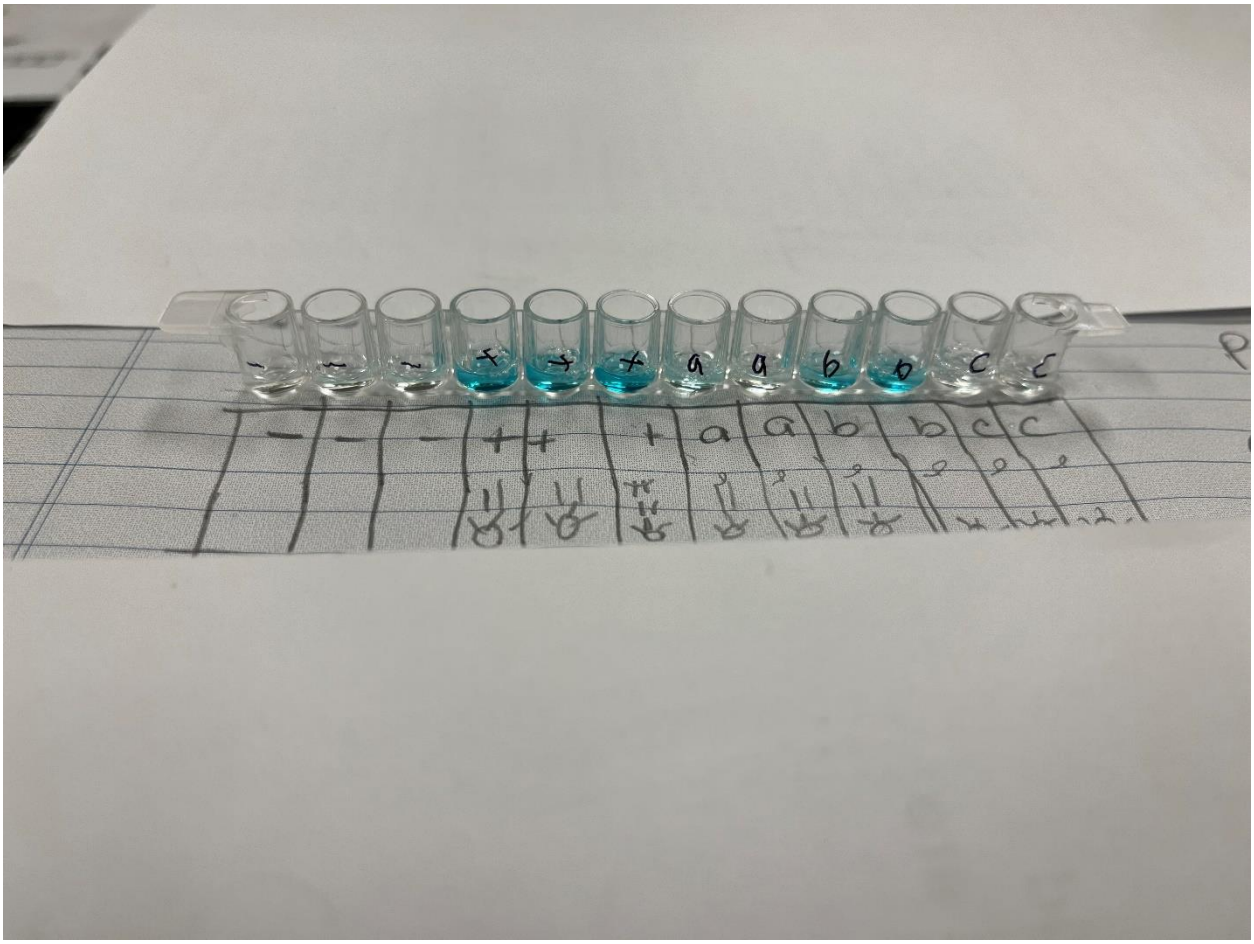
Results:



Discussion:

I enjoyed this activity, it required a steady hand in placing the purified antigen and the positive and negative control substance into each wells. As you can see from the photo above we accidently contaminated the first well of ‘a.’ Fortunetly all the other wells seemed to be good.

Conclusion:

I would do this experiment again. It was step by step informative and pretty simplictic to follow along with.