Laboratory 15: ELISA Antibody Test

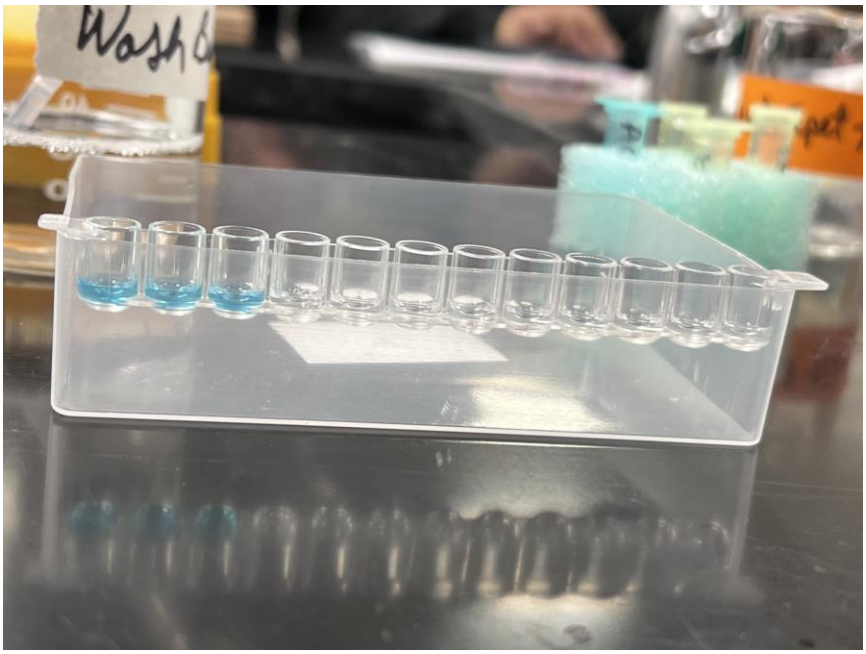
Purpose:

The purpose of laboratory 15 ELISA antibody test was to detect what antibodies and antigens were in our samples. This is done though different prepping steps as well as observing color change in the substances to know what is present in the wells.

Procedure:

- Label yellow tubes to identify testing samples.
- Label 12-well strip, 1-3: "+" for positive controls, 4-6: "-"for negative controls, and the remaining labeled with the sample being used.
- Use a fresh pipet tip to transfer 50 mL of purified antigen into the 12-wells.
- Wait 5 minutes for the antigen to bind with wells.
- WASH:
    - Tip micro-plate strip upside down on paper towels gently tapping the strip a few times. Avoid splashing back in wells.
    - Discard paper towel
    - Using transfer pipet, fill each well with wash buffer and copy steps from above.
    - Tip micro-plate strip upside down onto paper towels and tap.
    - Discard paper towels
- Repeat wash step.
- Using fresh pipet, transfer 50 mL of positive control into the 3 "+" wells.
- Using fresh pipet, transfer 50 mL of negative control into the 3 "-"wells.
- Transfer 50 mL of serum samples into each of the 3 wells, using fresh pipet.
- Wait 5 minutes for antibodies to bind to their targets.
- Wash the unbound primary antibody out of wells twice using wash technique.
- Use fresh pipet to transfer 50 mL of secondary antibody into each of 12 wells.
- Wait 5 minutes for antibodies to bind to their targets.
- Wash the unbound secondary antibody out of wells 3 times using wash technique.
- Use fresh pipet to transfer 50 m of enzyme substrate into 12-wells.
- Wait 5 minutes and observe/record results.

Results:



Left to right: **1-3**: "+" 4**-6**: "-"7**-12**:" serum."

Discussion:

This lab consisted of my partner and I adding a positive controls and negative controls to a micro plate, before being able to see any type of reaction we had to do several washes to the plate. The washes we had to do to the micro-plate threw my partner and I off, we at first thought our professor was trying to trick us until the end in which we actually got a reaction. After adding the last drops of the positive and negative controls we started to see a change in the positive controls, the change started to occur after about one minute after adding the positive solution.

Conclusion:

In conclusion I would say that I have a better view of antibodies, and their effects after multiple preparation steps. This lab was a learning experience, especially after adding and removing solutions on the micro-plate many times and having no constant solution or seeing any change in the micro-plate.