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BIO 125

12/5/23

Laboratory 15: ELISA Antibody Test

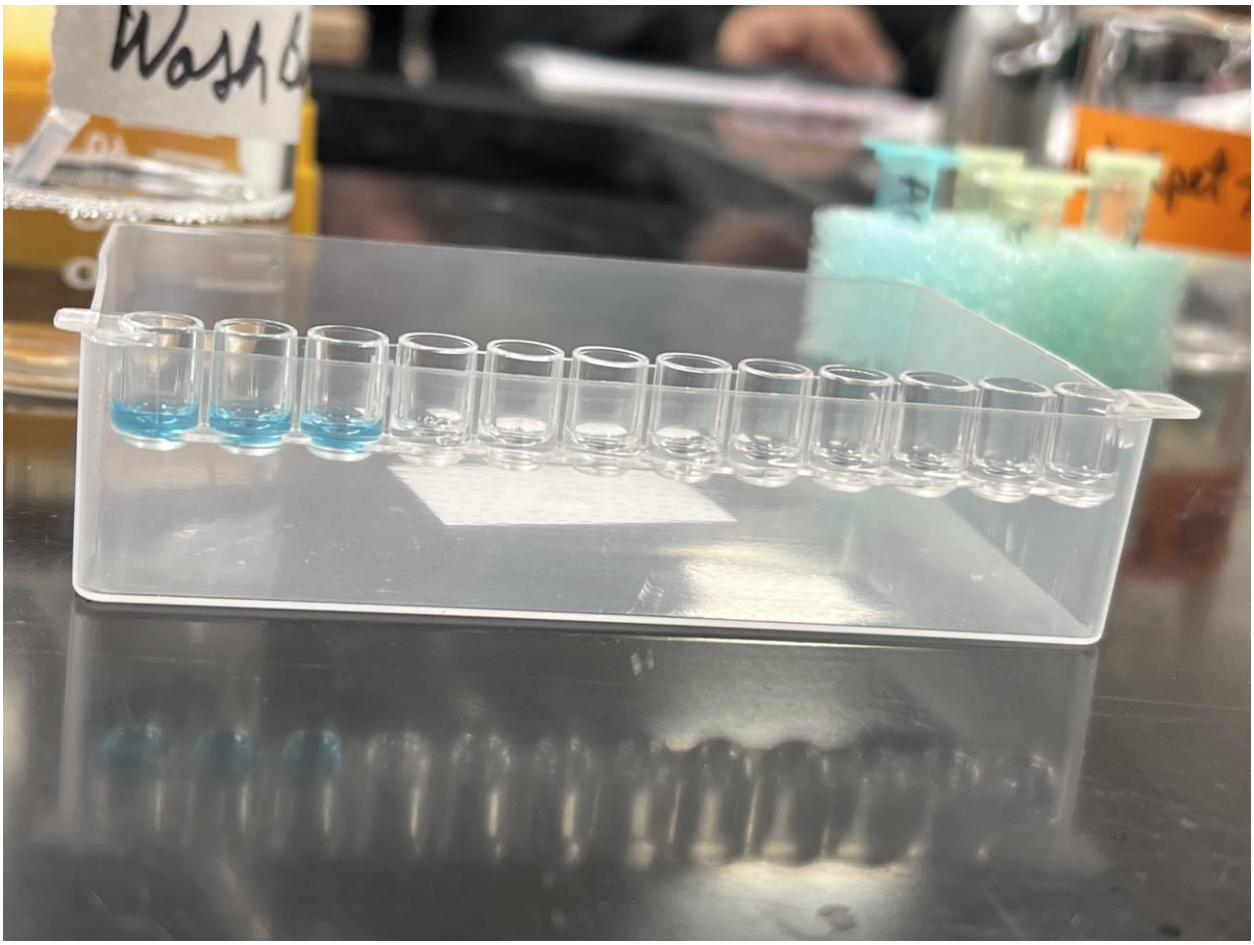
Purpose:

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 microplate 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 microplate 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:

In this lab we were testing to detect if there was a specific substance, according to the results the first 3 wells turned a blue color but the other wells remained clear. This indicates that the test detected the substance. So there were antibodies present in wells 1-3. I thought this lab was interesting in how it worked. At first it seemed odd to me that everything we would add to the wells ending up getting washed out, it confused me because I thought that we would mix everything together and finding out that binding was occurring made more sense. I thought that washing it with the buffer would just get rid of everything but I realized that’s just what makes it work more efficiently and it appears my results came out well. I compared my results with another lab group and had the same results but they said their color change took about 3 minutes to happen while ours happened almost immediately. I’m not sure if this had to do with how we washed our wells each time or just the serum we used.

Conclusion:

In conclusion, I found this lab to be confusing but then understood why the steps we did took place and were necessary to have efficient and accurate results which made the lab a fun

one and a more interesting one for me. It was nice to see how the antibodies were able to appear pretty much immediately and I got to learn a lot more about how people can detect antibodies in others.