Brisa Herrera

Laboratory 15- ELISA

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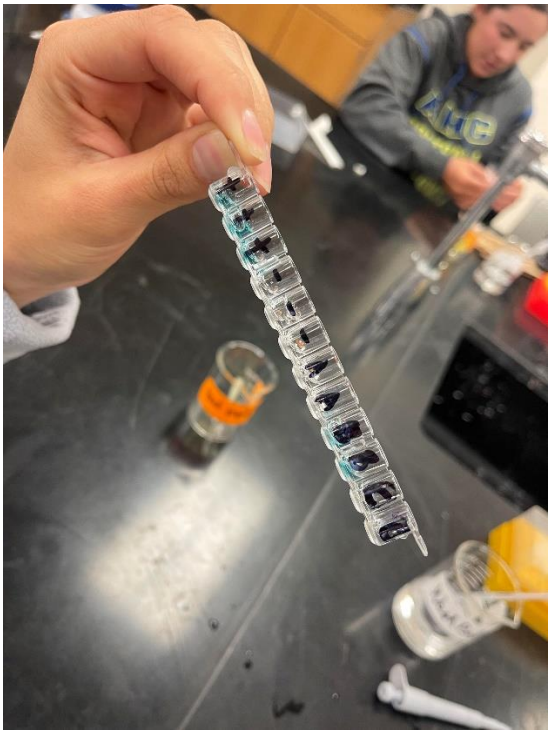
Purpose – The purpose of laboratory 15 ELISA, was to see how Antigen (AG), secondary antibody (SA), and enzyme substrate (SUB) reacted together after placing them in the wells and letting them bind together for a a good 10 minutes. The enzyme substrate was the solution that made a few of the wells turn a different color. Due to the wells turning a different color you can see that a few of the positive wells and negative wells did not bind to their antibodies due to a solution. A few of the "A" and "B" and "C" were not as reacting as the positive and negative wells.

Procedures – In laboratory 15 ELISA, we started off by labeling the yellow tubes (if necessary) to identify the samples being tested. We then labeled the 12-well strips. On each strip we labeled the first three wells with a "+" for the positive controls and the next three wells with a "-"for the negative controls and the remaining wells we label them sample A and sample B to identify the samples bring tested (three wells each). We used a fresh pipet tip to transfer 50 ul of purified antigen (AG) into each of the 12 wells of the microplate strips. We waited 5 minutes for the antigen to bind to the plastic wells. We then washed the 12 wells and made sure it has no antigen in them by tipping the microplate strip upside down onto the paper towels and gently tap on the strip a few times upside down. Make sure to avoid splashing sample back into wells. Then we use the transfer pipet to fill each well with wash buffer taking care not to spill over into neighboring wells. After that use a new paper towel to tip the strip upside down onto the paper towels. Then we repeat the wash step one more time. We use a fresh pipet to transfer 50 ul of the positive control (+) into the three "+" wells. Use a fresh pipet to transfer 50 ul of the negative control (-) into the three "-" wells. Transfer the 50 ul of each of your team's serum samples into each of the appropriately initialed three wells, using a fresh pipet for each serum sample. Wait 5 minutes for the antibodies to bind to their targets. After that we wash the unbound primary antibody out of the wells by repeating all the washing steps two times. After washing the wells twice we used a fresh pipet tip to transfer 50 ul of secondary antibody (SA) into each of the 12 wells of the microplate strips. We wait 5 minutes for the antibodies to bind to their targets. After, we wash the unbound secondary antibody out of the wells by repeating the wash steps three times. Use a fresh pipet tip to transfer 50 ul of enzyme substrate (SUB) into each of the 12 wells of the microplate strip. After this step we wait 5 minutes and record our results.

Results –



Discussion – Laboratory 15 ELISA, was a lab that I liked doing. Mixing the different type of substrates was fun because I honestly did not know what to expect in the beginning. Mixing AG and SA didn't do much to the wells, just a little bit of bubbling but I was not sure if that was due to how many times we were washing them. After adding SUB the wells started turning a different color. The wells that turned a light blue were three of the positive "+" and two of the "B" which was interesting because a few of the wells had the same antibodies in them. What was cool was seeing them turn a different color due to the SUB. I would have loved for the other wells to turn a different color as well. I really enjoy doing these type of labs because your mixing a lot of different chemicals together and you just don't know the ending results, which is always exciting to see. If I could do this lab all over again I would. Very thoughtful of Dr. Oakerbloom to have us do this lab. It was one of our last labs of the class which is very sad because I am going to miss this class. I really enjoyed coming to lecture and lab because he made it fun to

learn new material. Thank you Dr. Oakerbloom for always taking your time and being patient with us with new material and especially when we were lost in lab. Very few teachers a willing to help you but Dr. Oakerbloom was always there to help.

Conclusion – All in all, the purpose of laboratory 15 ELISA, was to see how Antigen (AG), secondary antibody (SA), and enzyme substrate (SUB) reacted together after placing them in the wells and letting them bind together for a a good 10 minutes. The enzyme substrate was the solution that made a few of the wells turn a different color. Due to the wells turning a different color you can see that a few of the positive wells and negative wells did not bind to their antibodies due to a solution. Mixing all the solutions together and using pipets were fun to do. We used 50 ul of the different solutions which I thought would fill the wells but it seemed as if the wells were sucking up all the liquid. A very fun lab that was done.