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Lab 15

12/1/2023

Title: ELISA

Purpose: To detect antigens in the

Procedure:

Label the 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). Use a fresh pipet tip to transfer 50 µl with purified antigen (Ag) into all 12 wells of the micro-plate strip. Wait 5 minutes for the antigen to bind to the plastic wells.

Wash

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. Discard the top paper towel. Use your pipet to fill each well with wash buffer, taking care not to spill over into neighboring wells. Note: the same pipet tip is used for all washing steps. Tip the microplate strip upside down onto the paper towels and tap.

Discard the top 2–3 paper towels. Repeat wash.

Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells. Transfer 50 µl of each of your team's serum samples into the appropriately initialed three wells, using a fresh

pipet tip for each serum sample.

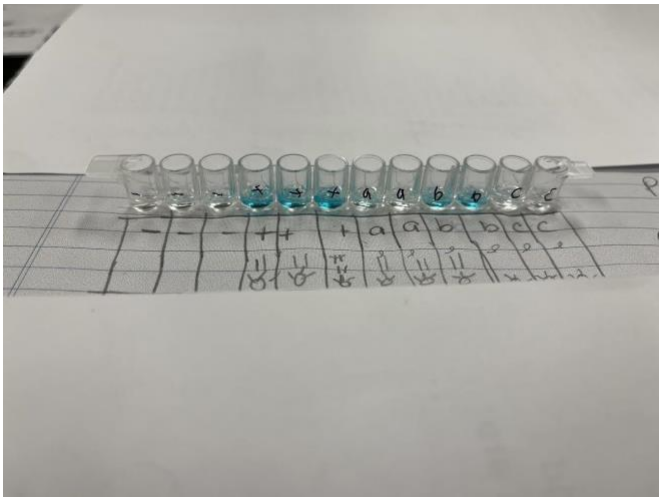
Wait 5 minutes for the antibodies to bind to their targets. Wash two more times.

Use a fresh pipet tip to transfer 50 µl of secondary antibody into all 12 wells of the microplate strip. Wait 5 minutes for the antibodies to bind to their targets. Wash three more times.

Use a fresh pipet tip to transfer 50 µl of enzyme substrate (TMB) into all 12 wells of the microplate strip. Wait 5 minutes.

A color change indicates that the serum contained specific antibodies which reacted against the original antigen.

Results:



Discussion: For lab 15 my lab partner and I got to see how antigens bind. We performed multiple steps to see which ones had bonded. Unfortunately, all of our tubes had been contaminated so every single one was blue. Another classmate let us use her photo for reference (Cynthia).

Conclusion: This was a fun little experiment to do for our last lab. Using purified antigen, positive and negative controls, secondary antibodies, and enzyme substrate we were able to see

how antigens bond. This experiment while easy, required lots of concentration. Also, the directions should be followed carefully; I had a few distractions and lost track of the tubes as I did not label them properly. I thoroughly enjoyed this lab and would love to try this one again in the future.