Biology 125- Human Physiology

Laboratory 15 – ELISA Antibody Test

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I. Purpose

ELISA stands for Enzyme, Linked, Immuno, Sorbent, Assay and is a common laboratory test method used to identify and quantify certain proteins, hormones, antigens, and antibodies in samples of body fluid. During this ELISA laboratory test we will be identifying whether or not the samples are positive for any antigens in the serum tubes.

II. Procedure

15: PROTOCOL III- ELISA Test

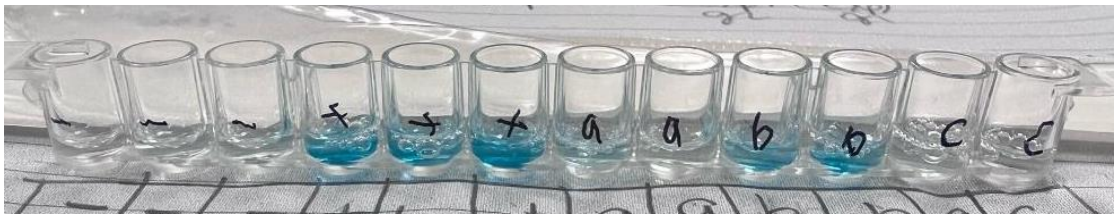
1. Label the yellow tubes (if necessary) to identify the samples being tested.
2. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "_" for the negative controls. Label the remaining wells to identify the samples being tested (3 wells each).
3. Use a fresh pipet tip to transfer 50 u' 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.
    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.
7. Use a fresh pipet tip to transfer 50 ul of the positive control (+) into the three "+" wells.
8. Use a fresh pipet tip to transfer 50 ul of the negative control (-) into the three "_" wells.

9. Transfer 50 ul 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.
12. Use a fresh pipet tip to transfer 50 ul 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.
15. Use a fresh pipet tip to transfer 50 ul of enzyme substrate (SUB) into each of the 12 wells of the microplate strip.
16. Wait 5 minutes. Observe and record the results.

III. Result

15: PROTOCOL III- ELISA Test

(-)	(-)	(-)	(+)	(+)	(+)	(a)	(a)	(b)	(b)	(c)	(c)
Not Present	Not Present	Not Present	Present	Present	Present	Not Present	Not Present	Present	Present	Not Present	Not Present



IV. Discussion

All negative (-) wells were negative for the antigen and the positive (+) wells were positive which means our controls were accurate. After completing the A, B, and C wells only the B wells came back positive. As you can see from the photo above the first A well is slightly blue due to contamination during the wash step.

V. Conclusion

This experiment proved to be very interesting being able to tell if an antigen is present in a serum or plasma. I learned that it is very important not to cross contaminate because it can skew results, especially if you are unaware of cross contamination.