

### ELISA Antibody Test

Purpose: Experiment with the ELISA antibody test.

#### Procedures:

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 µl 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 µl of the positive control (+) into the three "+" wells.
8. Use a fresh pipet tip to transfer 50 µl of the negative control (-) into the three "-" wells.
9. Transfer 50 µl 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 µl 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 µl of enzyme substrate (SUB) into each of the 12 wells of the microchip strip.
16. Wait 5 minutes. Observe and record the results.

#### Results:



Discussion: The main mistake that couldve been made was tipping over the test strips, luckily that did not happen to our group, so our results are accurate.

Conclusion: To conclude, the antibodies in our test strip turned a lightish blue. No mistakes were made with our group of four.