

Purpose

Laboratory 15 experimented with AG's, SA's and SUB's. Antigens are substances that cause the immune system to produce antibodies against it. Secondary antibodies are antibodies that indirectly detect a primary antibody that specifically binds to the protein of interest. An enzyme substrate is a molecule that is acted upon by an enzyme. The purpose of laboratory 15 was to see how antigens (AG's), secondary antibodies (SA) and enzyme substrates (SUB) bind to positive and negative wells.

Procedure

First we labeled the yellow tubes to identify the samples being tested, then we labeled our 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. After we labeled the remaining wells to identify the samples being tested. We used a fresh pipet trip to transfer 50 ul of purified antigen into each of the 12 wells of the microplate strip, we waited 5 minutes for the antigen to bind to the plastic wells. We then washed the microplate strip. Next we used a new fresh pipet tip to transfer 50 ul of the positive control into the three + wells and with another fresh pipet tip we transferred 50 ul of the negative control to the - wells. After that we transferred 50 ul of each of your team's serum samples into each of the appropriately initial 3 wells using a fresh pipet for each serum sample. We waited 5 minutes for the antibodies to bind to their targets. We then washed the microplate strip again. Next, we used a fresh pipet tip to transfer 50 ul of secondary antibody into each of the 12 wells of the microplate strip. We waited 5 minutes for the antibodies to bind to their targets. We then washed the microplate strip again. Lastly we used a fresh pipet tip to transfer 50 ul of enzyme substrate into each of the 12 wells of the microplate strip. We waited 5 minutes and recorded the results.

Results



Discussion

The results of the experiment were interesting to look at and see the change on the antibodies. . The first three positive wells all turned into a light blue substance. The three negative wells had no change to them. I noticed the substance that had the biggest effect on the wells was when we transferred enzyme substrate to the wells. I really liked this lab because it was interesting to how antigens bind to antibodies. An experimental error that may have occurred would have been if we were not washing the microplate strip correctly after every step because it would have given inaccurate results. This could be avoided by being more careful and thoughtful when washing the strip.

Conclusion

To conclude this experiment, all antibodies react differently to antigens, secondary antigens and enzyme substrates. Enzyme substrates had more of a reaction than antigens and secondary

antigens on the antibodies. I really enjoyed this lab and was able to see how the antigens bind to antibodies. Thank you DR oak.