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Formative Assessment Answer Key

Modeling an Enzyme-Linked Immunosorbent Assay

1. How do you think capture antibodies attach to the plate during the incubation period?
   Answers will vary: Proteins contain chemical groups that enable them to bind to various materials. Advanced students (such as those in AP or honors classes) may mention specific chemical interactions, such as hydrogen bonds, hydrophobic interactions, or ionic bonding, when discussing how proteins bind to different materials. The goal of this question is to spark discussion; while it is true that antibodies bind to the polymer primarily through hydrophobic interactions, it is not essential for students to identify these specific types of chemical interactions.

2. Why do you think a patient's blood plasma may have many different kinds of antigens?
   Antigens are proteins that trigger an immune response. Students' answers may vary but could include the following points:
   - A person can be exposed to multiple pathogens simultaneously, with each pathogen possessing a unique antigen.
   - We encounter various foreign proteins from environmental sources, including pollen, viruses, bacteria, fungi, and more. Our immune system can respond to many of these antigens at the same time.

3. Can the ELISA well you prepared identify all of the various kinds of antigens present in the blood of the patient? Explain your answer.
   No. Students should note that while our bodies have many different antibodies for many different antigens, the ELISA plate has only one type of antibody to be able to recognize ONE type of antigen.

4. A very important step performed in real ELISAs is to wash the plates between each incubation period. Why do you think it is necessary to wash the ELISA plates several times as part of the assay?
   Washing the plates removes unbound molecules. Capture antibodies should be immobilized on the surface of the plate; excess ones that do not bind to the plate need to be removed.

   When other components are added (secondary or detection antibody, enzyme, substrates), they should bind to the immobilized antibody. Washing removes reagents that do not bind. If unbound reagents are left on an ELISA plate, they will produce a signal and yield a false positive.

5. Describe how the negative control and positive control differ from one another.
   The negative control does not include the antigen, so no signal or color should develop. The positive control has the antigen, and a color should develop.

6. Why is it important to run controls? Controls help us determine whether the test results are valid. If a positive control does not yield a color, or if a negative control yields a color, we can conclude that something in the experiment has gone wrong and the results cannot be trusted.

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7. Describe one mistake in the experiment that would lead to a FALSE negative.
   In a false negative, color DOES NOT develop where there should be a color.
   A positive control well without a color shows a false negative. A color fails to develop if:
   - Reagents are non-viable (too old, inactive).
   - Enzyme was not added.
   - Substrate was not added.
   - Reagents are washed away before they are immobilized due to improper incubation period.

8. Describe a mistake that would lead to a FALSE positive.
   In a false positive, a color develops where there should be no color. A negative control that changes color is considered a false positive. False positives can happen if:
   - Wells were contaminated with antigen.
   - Wells were not properly washed, and therefore **unbound** enzymes or antibodies stay behind and react in the well.
   - Antibodies bind nonspecifically to other molecules in the blood serum.