BI 207 Spring 2020 - Lab 5 ELISA Simulation

Introduction

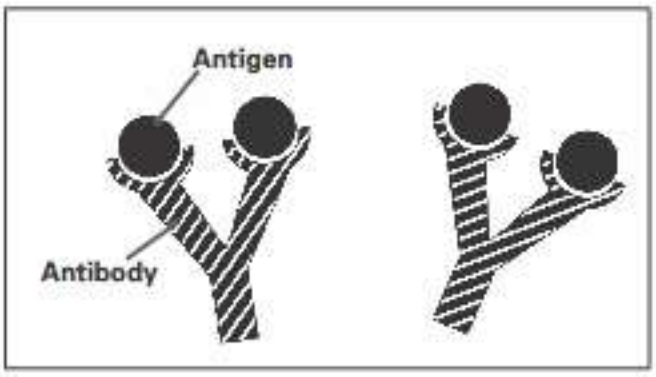
The body possesses several lines of defense against infection by pathogenic organisms. Pathogens are tiny, disease-causing agents including viruses, bacteria, protozoa, molds, and other microorganisms. Pathogens invade the body and multiply; they can cause sickness or even the death of the invaded individual. The body employs three lines of defense to prevent and fight off such dangerous intrusions. The first two defense modes are nonspecific. They include the body's physical barriers and the nonspecific immune system. These defenses function without regard to the type of pathogenic intruder. The third layer of defense is the body's specific immune system. Specific immune responses are tailored to the type of invading pathogen.

Specific Immune Response

Specific immune responses are triggered by antigen molecules. Antigens include proteins and other molecules produced by pathogens. The key players in the specific immune defense are dendritic cells, macrophages, and small white blood cells called B lymphocytes (B cells) and T lymphocytes (T cells).

Phagocytic macrophages and dendritic cells break down pathogens and display antigenic fragments from the pathogens on the surface of their cell membranes. B and T lymphocytes circulate through the body in the blood and lymph. When T cells see displayed antigenic fragments, they stimulate specific B cells to reproduce and generate antibodies designed against the specific structure of the antigen encountered. Thus, the word *antigen* is derived from the term "antibody generator."

Antibodies are a group of serum proteins (also referred to as immunoglobulins) that are found in



the bloodstream or bound to cell membranes. These proteins all have the same basic Y-shaped structure, but have different antigen binding sites at their ends. Antigen binding sites are designed to fit the shape of specific antigens. Antibodies bind to antigens like a lock and key, forming antigen-antibody complexes (see Figure 1).

Figure 1. Antigen-antibody complex

When an antibody forms an antigen-antibody complex, generally it marks the invading organism/antigen for destruction or for clearance from the bloodstream by phagocytic cells. This removal is designed to prevent the organism/antigen from infecting the cell. Antigen-antibody complexes also stimulate additional immune responses to aid the body in clearing an infection.

ELISA

Scientists have applied the basic principles of antibody-mediated immunity to an assay for detecting infection by specific organisms. This assay is called an ELISA (enzyme-linked immunoabsorbant assay) and is based on the principle that antibodies produced in response to pathogens attach to their antigen targets with great specificity to form antigen-antibody complexes.

There are two types of ELISA tests—direct ELISA and indirect ELISA. Indirect ELISA is used to detect infection by testing patients' blood for the presence or absence of antibodies against a particular pathogen. The presence of such antibodies indicates that the individual has been infected and that their body has launched an immune response against the disease-causing agent.

In the first step of an indirect ELISA, antigen proteins purified from the infectious agent, or genetically engineered versions of the antigens, are added to the wells of plastic microtiter plates. These antigen proteins bind to the bottom of the well by forming hydrophobic associations with the plastic surface (see Figure 2a). The wells of the plate are then washed with a buffer to remove any unbound material.

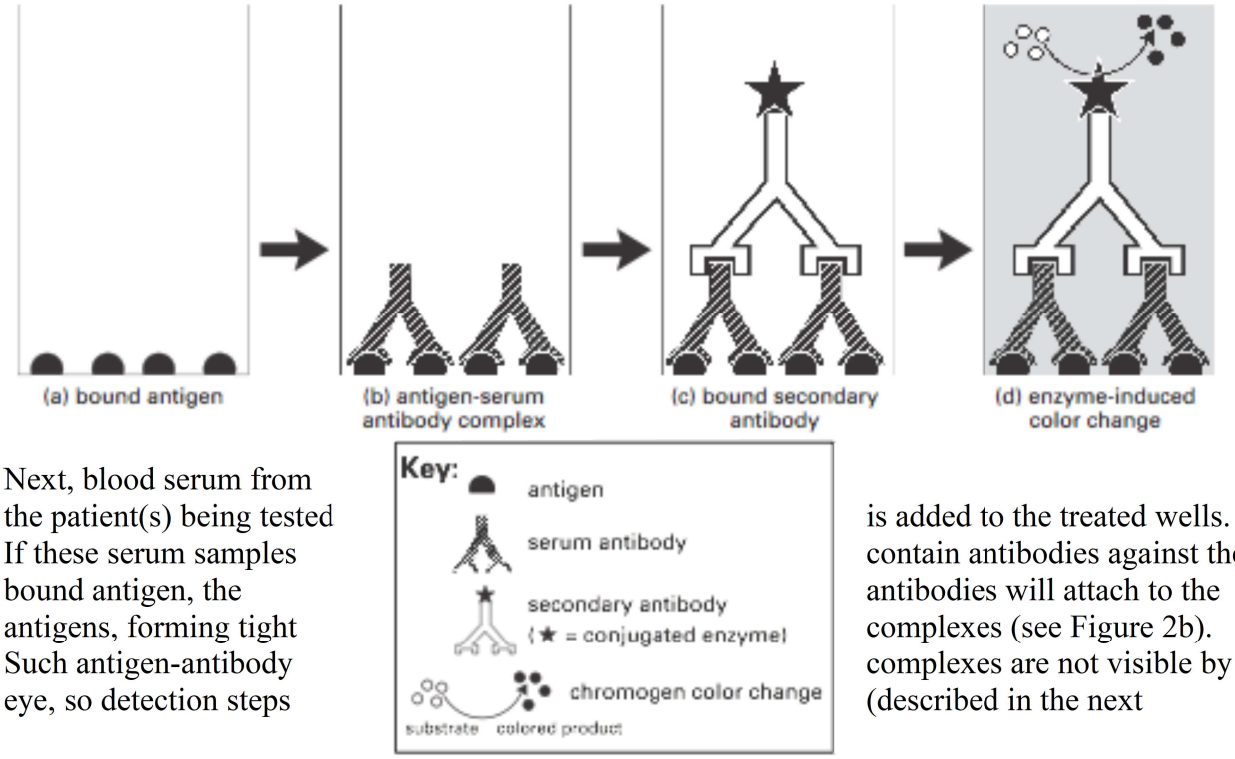


Figure 2. ELISA schematic of a positive result

Next, blood serum from the patient(s) being tested is added to the treated wells. If these serum samples contain antibodies against the bound antigen, the antibodies will attach to the antigens, forming tight complexes (see Figure 2b). Such antigen-antibody complexes are not visible by eye, so detection steps (described in the next paragraph) must be employed to visualize them. The wells are again washed to remove any unbound proteins.

Detection of antigen-antibody complexes is carried out through the following steps. A secondary antibody that recognizes antibodies produced by humans (anti-human antibody) is added to the wells. If antigen-antibody complexes formed in the wells, this secondary antibody recognizes and binds to the primary antibodies from the patients' serum (see Figure 2c). The secondary antibody is attached to an enzyme that will facilitate the final detection. (This antibody/enzyme combination is called a conjugate.)The wells are rinsed one last time to remove unbound molecules.

In the final step, a chromogen substrate is added to the wells of the plate. If present, the enzyme that is linked to the secondary antibody facilitates a chemical reaction that changes the color of the chromogen (see Figure 2d). A color change indicates that the patient possesses antibodies to the antigen and has been infected. No change in color indicates that the patient has not been infected, or that their body has not yet launched an immune response to produce antibodies against the invading antigen. Positive results determined by ELISA undergo a different test to confirm the findings.

In this simulated assay, each sample will be tested in triplicate to ensure reproducibility. Known positive and negative samples are included as controls. For ease of performance, the well washing steps of a true ELISA have been eliminated in this simulation. It is important to note

that the washes are a necessary step of an actual assay. Likewise, it is critical to use a clean pipet for each new sample or reagent to prevent cross-contamination of the wells.

Instructions

1. Using one plastic pipet, carefully administer 3 drops of simulated antigen in each well of rows A and B of the microtiter plate. Discard the pipet after use. In a true ELISA assay, the antigen would bind to the bottom of the microtiter plate. The wells would then be washed with a buffer to remove any unbound molecules. In this simulated lab activity, the washing step has been eliminated.

2. Using a clean pipet, add 3 drops of positive control to wells A1, A2, and A3 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

3. Using a clean pipet, add 3 drops of negative control to wells A4, A5, and A6 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

4. Using a clean pipet, add 3 drops of Patient A sample to wells A7, A8, and A9 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

5. Using a clean pipet, add 3 drops of Patient B sample to wells A10, A11, and A12 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

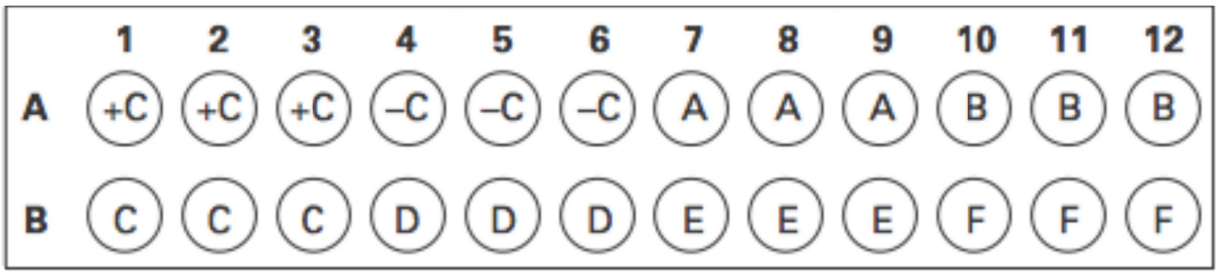
6. Using a clean pipet, add 3 drops of Patient C sample to wells B1, B2, and B3 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

7. Using a clean pipet, add 3 drops of Patient D sample to wells B4, B5, and B6 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

8. Using a clean pipet, add 3 drops of Patient E sample to wells B7, B8, and B9 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

9. Using a clean pipet, add 3 drops of Patient F sample to wells B10, B11, and B12 of the microtiter plate (see Figure 3). Do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use. In a true ELISA assay, at this point, the wells would be washed with a buffer to remove any molecules that have not bound to the adhered antigen. In this simulated lab activity, the washing step has been eliminated.

10. Using a clean pipet, add 3 drops of simulated secondary antibody to each well of rows A and B on the microtiter plate. Keep track of each well as the reagent is added and do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use. In a true ELISA, the wells would again be washed to remove unbound molecules. This step has been eliminated from this simulation.



+C = positive control, –C = negative control, A–F = Patients A–F

Figure 3. Microtiter plate sample wells

11. Using the remaining clean pipet, add 3 drops of simulated chromogen to each well of rows A and B on the microtiter plate. Keep track of each well as the reagent is added and do not allow the pipet to touch the liquid already in the wells. Discard the pipet after use.

12. All the reaction wells will turn light green when the chromogen is added. A change from light green to purple indicates a positive result. Incubate the microtiter plate at room temperature for a minimum of 5 minutes and a maximum of 10 minutes to allow for color development.

13. After 5–10 minutes of incubation, record your results in the data table. Compare the color of each patient sample to that of the positive and negative control. Colored results that are in between the positive and negative control should be scored as weak positives.