Using ELISA Assay to Track Disease Outbreaks

Overview

In this experiment, you will track a SIMULATED disease outbreak. You will perform an ELISA or enzyme linked immunosorbent assay to determine if you have been exposed to a contagious "disease". The ELISA uses antibodies to detect the presence of a disease agent, for example, viruses, bacteria or parasites in your blood or other bodily fluid. You will then track the disease back to its source. It is important to emphasize that this is a SIMULATED lab, that is, you are NOT working with any pathological agents that cause disease.

Introduction

When you are exposed to a disease agent, your body displays an immune response. Molecules that cause your body to display an immune response are called antigens, and may be any molecules, including components of infectious agents like bacteria, viruses, and fungi. After exposure, within days, millions of antibodies—proteins that recognize the antigen and bind very tightly to it—are circulating in your bloodstream. The antibodies seek out and attach themselves to their target antigens, flagging the invaders for destruction by other cells of the immune system.

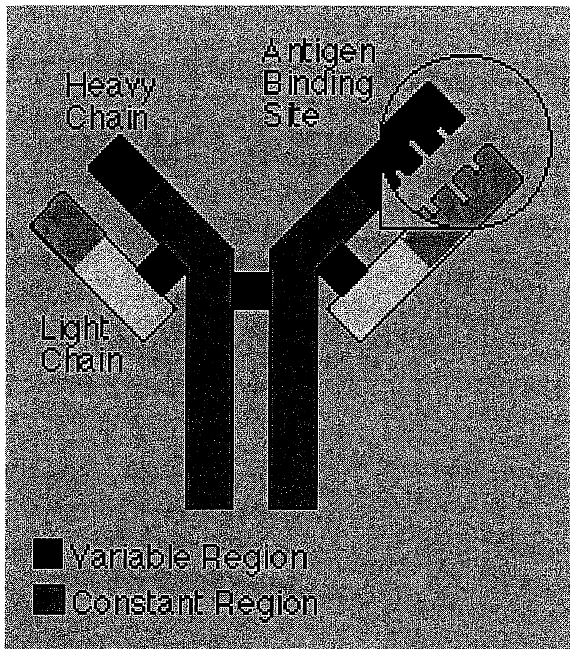
Over 100 years ago, biologists found that animals' immune systems respond to invasion by "foreign entities " or antigens. Today, antibodies have become vital scientific tools, used in biotechnology research and to diagnose and treat disease. The number of different antibodies circulating in the blood has been estimated to be between 10^6 to 10^{11} so there is usually an antibody ready to deal with any antigen. In fact, antibodies make up to 15% of your total blood serum protein. Antibodies are very specific; each antibody recognizes only a single antigen.

Scientists have learned to use the immune response of animals to make antibodies that can be used as tools to detect and diagnose diseases. Animals such as chickens, goats, rabbits and sheep can be injected with an antigen, and, after a period of time, their serum will contain antibodies that specifically recognize that antigen. If the antigen was a disease agent, the antibodies can be used to develop diagnostic tests for the disease. In an immunoassay, the antibodies used to recognize antigens like disease agents are called primary antibodies; primary antibodies confer specificity to the assay.

Secondary antibodies are made in the same way. In an immunoassay, secondary antibodies recognize and bind to the primary antibodies, which are antibodies from another species. Secondary antibodies are prepared by injecting antibodies made in one species into another species. It turns out that antibodies from different species are different enough from each other that they will provoke an immune response. For example, to make a secondary antibody that will recognize a human primary antibody, human antibodies can be injected into an animal like a rabbit. After the rabbit mounts an immune response, the rabbit serum will contain antibodies that recognize and bind to human antibodies Secondary antibodies are conjugated to an enzyme such as horseradish peroxidase (HRP) that produces a blue color in the presence of its substrate, TMB. These antibody and enzyme tools are the basis for the ELISA.

ELISA is used every day in medicine and agriculture. The ELISA method is used for home pregnancy tests, disease detection in people, animals, and plants, detecting illegal drug use, testing indoor air quality and determining if food is labeled accurately. For new and emerging diseases like severe acute respiratory syndrome (SARS), one of the highest priorities of the US centers for Disease Control (CDC) and the World Health Organization (WHO) has been to develop an ELISA that can quickly and easily verify whether patients have been exposed to the virus.

Positive and negative controls are critical to any diagnostic tests. Control samples are necessary to be sure your ELISA is working correctly. A positive control is a sample known to be positive for the disease agents, and a negative control is a sample that does not contain the disease agent. In this lab, you will be performing an ELISA. You will be given a simulated sample that you will share with your class. One or two of the samples in the class have been "infected". You will also be provided with positive and negative control samples. Then your class will assay your samples for the presence of the disease agent to track the spread of the disease through your class population.



Antibody

Each antibody is made up of two identical heavy chains and two identical light chains, shaped to form a Y.

The sections that make up the tips of the Y's arms vary greatly from one antibody to another; this is called the variable region. It is these unique contours in the antigen-binding site that allow the antibody to recognize a matching antigen, much as a lock matches a key.

The stem of the Y links the antibody to other participants in the immune defenses. This area is identical in all antibodies of the same class—for instance, all IgEs—and it's called the constant region.

http://press2.nci.nih.gov/sciencebehind/immune/immune10.htm

The Scenario

Students have been on a field trip. While on the public PAT bus, it is possible that they have been exposed to smallpox, deliberately released in aerosol form. NOTE: smallpox virus, if not exposed to Uv light, may survive for 24 hours in a cool, dry location.

It is important to determine as soon as possible which students have been exposed. Vaccination within 2 – 3 days of exposure can prevent smallpox, but

the vaccine is in short supply. Also, vaccination can have nasty side effects, so no one should be vaccinated unnecessarily. Vaccination of exposed individuals is essential to prevent further spread of the disease.
To determine which students have been exposed, perform an ELISA to detect the virus in samples of their bodily fluid.

Students who test positive for the presence of the virus should undergo immediate vaccination. NOTE: Since smallpox was eradicated globally in 1977, when the threat of smallpox as a weapon arose there was no ELISA in place to detect exposure. However, ELISAs to detect the virus and the immune response to the virus are under development.

PROTOCOL

1. SHARE your Samples (simulated of bodily fluids)

Label each eppindorf tube with your initials. These are your bodily fluid samples that will be shared with your classmates.

2. Using a plastic transfer pipet, ~~find another student and use the pipet to transfer all 750µl of your~~ sample into the tube of the other student (It doesn't matter whose tube is used to mix both samples), Gently mix the samples by pipetting the mixture up and down. Then, take back half of the shared sample (about 750µl) to your own tube. Write down the name of that student next to Sharing Partner #1:

Sharing Partner #1______________________________________

Sharing Partner#2______________________________________

Sharing Partner #3______________________________________

3. Repeat the sharing protocol two more times with 2 other students so that you have shared your sample with 3 other students total. Make sure that you record their names in the order in which you shared.

Perform the ELISA

4. Label the outside wall of each well of your 12 well strip. Each group of 2 will share a strip of 12 wells. On each strip label the first three wells with a "+" for the positive controls and the next three wells with a "-" for the negative controls. On the remaining wells, write you and your partners' initials and sample number.

5. Bind the antigen to the wells: Use a pipet to transfer 50 µl of the positive control (+) into the 3 wells.

6. Use a fresh pipet tip to transfer 50 μl of the negative control (-) into the 3 wells.

7. Use a fresh pipet tip for each sample and transfer 50 μl of each of your samples into the labeled wells. Your samples contain many proteins and may or may not contain the disease agent (antigen).

8. Wait 5 minutes while all the protein in the samples bind to the plastic wells. ELISA is called an immunosorbent assay because proteins adsorb (bind) to the plastic wells.

9. Place the strip on top of several paper towels. Wash the unbound sample out of the wells by tipping the microplate strip upside down onto the paper towels to that the samples drain out, then gently tap the strip a few times upside down on the paper towels. Discard the top paper towel.

10. Use a pipet filled with wash buffer from the beaker to fill each well.

11. Tip the microplate strip upside down onto the paper towels so that the wash buffer drains out, then gently tap the strip a few times upside down on the paper towels. Discard the top 2 – 3 paper towels.

12. Use a fresh pipet to transfer 50 ul of primary antibody (PA) from the tube into all 12 wells of the microplate strip.

13. Wait 5 minutes for the primary antibody to bind. The primary antibody will seek out the antigen from the many proteins bound to the well. If your sample was "infected", the antibodies will bind tightly to the disease agent(antigen) in the wells.

14. Wash the unbound primary antibody out of the wells by repeating wash steps 9 – 11.

15. Use a fresh pipet tip to transfer 50 μl of secondary antibody (SA) from the tube into all 12 wells of the microplate strip.

16. Wait 5 minutes for the secondary antibody to bind. You detect the bound antibodies with HRP labeled secondary antibody. If the primary antibodies have bound to the antigen, the secondary antibodies will bind tightly to the primary antibodies.

17. Wash the unbound secondary antibody out of the wells by repeating steps 9 –11. This was step is done twice.

The secondary antibody is attached to an enzyme (HRP) that chemically changes the enzyme substrate, turning it from a colorless solution to a blue solution.

18. Use a fresh pipet tip to transfer 50 μl of enzyme substrate (SUB) from the brown tube into all 12 wells of the microplate strip.

19. Wait 5 minutes. If the disease antigen was present in your sample, the wells will turn blue. This is a positive diagnosis. If the wells remain colorless, the disease antigen was not present in your sample and the diagnosis is negative. Observe and record your results here:

Sample	Positive or Negative
1	
2	
3	
4	
5	
6	
Your sample	
Your sample	
Your sample	
Partner sample	
Partner sample	
Partner sample	

You can track the progress of the disease through the class by having each student record whether they tested positive or negative next to the names of the people with whom they shared "bodily fluids".

CLASS RESULTS

Student Name	***Positive or Negative***	***Sharing Partner***

Questions to answer:

1. The samples that you added to the microplate strip contain many protein and may or may not contain the disease antigen. What happened to the proteins in the plastic well if the sample contained the antigen? What if it did not contain the antigen?

2. Why did you need to wash the wells after every step?

3. When you added primary antibody to the wells, what happened if your sample contained the antigen? What if it did not contain the antigen?

4. When you added secondary antibody to the wells, what happened if your sample contained the antigen? IF it did not contain the antigen?

5. If the sample gave a negative result for the disease-causing agent, does this mean that you do not have the disease? What reasons could there be for a negative result when you actually do have the disease?

6. Why did you assay your samples in triplicate?

7. What antibody-based tests can you buy at your local pharmacy?

8. If you tested positive for disease exposure, did you have direct contact with one of the original infected students? If not, what conclusions can you reach about transmissibility of disease in a population?