

ELISA LAB

INTRODUCTION

Much of following is taken from BioRad's educational materials:

You are about to perform an experiment in which you will share simulated body fluids with your classmates. After sharing, you will perform an enzyme-linked immunosorbent assay or ELISA 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 body fluids. You will then track the disease back to its source.

When you are exposed to a disease agent, your body mounts an immune response. Molecules that cause your body to mount an immune response are called antigens, and may include components of infectious agents like bacteria, viruses, and fungi. Within days, millions of antibodies, proteins that recognize the antigen and bind very tightly to it, are circulating in your bloodstream. Like magic bullets, 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 animal 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 and 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. Other kinds of antibody tools, called 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 be recognized as foreign proteins and 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. The secondary antibodies used in this experiment are conjugated to the enzyme horseradish peroxidase (HRP) which produces a blue color in the presence of its substrate, TMB. These antibody and enzyme tools are the basis for the ELISA.

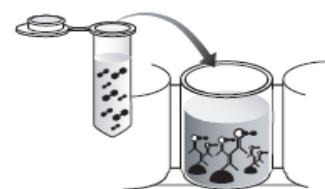
With its rapid test results, the ELISA has had a major impact on many aspects of medicine and agriculture. ELISA is used for such diverse purposes as pregnancy tests, disease detection in people, animals, and plants, detecting illegal drug use, testing indoor air quality, and determining if food is labeled accurately. For new and emerging diseases like severe acute respiratory syndrome (SARS), one of the highest priorities of the 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.

Some tests give positive or negative results in a matter of minutes. For example, home pregnancy dipstick tests are based on very similar principles to ELISA. They detect levels of human chorionic gonadotropin (hCG), a hormone that appears in the blood and urine of pregnant women within days of fertilization. The wick area of the dipstick is coated with anti-hCG antibody labeled with a pink compound. When the strip is dipped in urine, if hCG is present it will bind to the pink antibody, and the pink hCG-antibody complex will migrate up the strip via capillary action. When the pink complex reaches the first test zone, a narrow strip containing an unlabeled fixed anti-hCG antibody, the complex will bind and concentrate there, making a pink stripe. The dipsticks have a built-in control zone containing an unlabeled fixed secondary antibody that binds unbound pink complex (present in both positive and negative results) in the second stripe. Thus, every valid test will give a second pink stripe, but only a positive pregnancy test will give two pink stripes.

EXCHANGING OF FLUIDS AND ELISA

You will be provided the tools and an experimental protocol to perform an ELISA. You will be given a simulated body fluid sample that you will share with your classmates. One of the samples in the class has been “infected”. You will also be provided with positive and negative control samples. Then you and your fellow students will assay your samples for the presence of the “disease agent” to track the spread of the disease through your class population. The assay will involve:

- 1) Addition of your shared sample plus control samples to the wells in a microplate strip. Incubation will allow all the proteins in the samples to bind to the plastic wells via hydrophobic interaction. This is an immunosorbent assay because proteins adsorb (bind) to the plastic wells.
- 2) Addition of anti-disease antibody (primary antibody) to the wells. 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.
- 3) Detection of 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.
- 4) Addition of enzyme substrate to the wells. 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.



MATERIALS

- Yellow tubes - Student samples (0.75 ml) (1 per student)
- Violet tube - Positive control (0.5 ml)
- Blue tube - Negative control (0.5 ml)
- Green tube - Primary antibody (1.5 ml)
- Orange tube - Secondary antibody (1.5 ml)
- Brown tube - Enzyme substrate (1.5 ml)
- 12-well microplate strips
- 20-200 μ l adjustable micropipet
- Yellow tips
- Disposable plastic transfer pipets
- 70-80 ml wash buffer in beaker

PROCEDURE: EXCHANGING OF FLUIDS

1. Write down the number on your yellow tube at the top of your data sheet. These are your “body fluid” samples that will be shared randomly with your classmates.
2. When you are told to do so, find another student and use a plastic transfer pipet to transfer all of your sample into the tube of the other student. (It does not 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 750 μ l) to your own tube.
3. Write down the name and ID# of the student you just exchanged with on the data sheet for “Round 1.” Do not go on to the next round until the entire class is done with this round.
4. When you are told to do so, do round 2. Stop and wait until everyone is done with round 2 before going on to round 3.

PROCEDURE: USING ELISA TO TEST IF YOU CONTRACTED A DISEASE

1. Work in groups of 2.
2. Label the outside wall of each well of your 12-well strip. Two students may 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 your and your partner’s initials. For example, Florence Nightingale and Alexander Fleming would label their shared strip like this:



3. Bind the antigens to the wells: Transfer 50 μ l of the positive control antigen from the violet tube into the three “+” wells. Use a fresh tip and transfer 50 μ l of the negative control antigen from the blue tube into the three “-” wells. Use a fresh tip and transfer 50 μ l of your and your partner’s samples into the initialed wells.
4. Let stand for 5 min to allow antigens to bind to wells.
5. Wash the unbound samples out of the wells: Tip the microplate strip upside down onto the paper towels so that the samples drain out, then vigorously tap the strip upside down on the paper towels. Make sure to avoid samples splashing back into wells. Discard paper towel.
6. Use a fresh transfer pipet filled with wash buffer from the beaker to fill each well with wash buffer taking care not to spill over into neighboring wells. The same transfer pipet will be used for all washing steps. Tip the microplate strip upside down onto the paper towels so that the wash buffer drains out, then vigorously tap the strip a few times upside down on the paper towels.
7. Repeat step 6. Discard paper towels.
8. Use a fresh pipet tip to transfer 50 μ l of primary antibody (PA) from the green tube into all 12 wells of the microplate strip.
9. Wait 5 minutes for the primary antibody to bind.
10. Wash the unbound primary antibody out of the wells by repeating wash step 6 two times.
11. Use a fresh pipet tip to transfer 50 μ l of secondary antibody (SA) from the orange tube into all 12 wells of the microplate strip.
12. Wait 5 minutes for the secondary antibody to bind.

13. Wash the unbound secondary antibody out of the wells by repeating wash step 6 three times. Use a fresh pipet tip to transfer 50 μ l of enzyme substrate (SbB) from the brown tube into all 12 wells of the microplate strip.
14. Wait 5 minutes but not more than 20 minutes. Observe and record your results. A blue color means a positive reaction, while no change means negative.
15. We will analyze the results of the class data below to figure out who was first infected.

DATA SHEET

Your ID Number: _____

Are you infected? Circle **YES** or **NO**

Names and ID#s of those you exchanged:

Round 1 _____ ID#: _____

Round 2 _____ ID#: _____

Round 3 _____ ID#: _____

ID Number and Name of those infected	Contacts		
	Round 1	Round 2	Round 3
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
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