



Disease Epidemic – ELISA Technique

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Introduction

Epidemiology is the study of disease origin and transmission. This experiment allows students to experience a small scale “epidemic”, demonstrating the ease with which disease organisms are spread and enabling them to determine the originator of the disease and route of transmission.

A hypothetical contagious disease is spread in a population through the sharing of simulated body fluids. The students will then perform an ELISA test (Enzyme-Linked ImmunoSorbant Assay) to detect disease-causing agents in a simulated sample of their body fluid. Students will attempt to determine the originator of the epidemic, and again test samples to verify this hypothesis.

Objectives

1. To demonstrate the development and spread of an epidemic through a population.
2. To understand the importance of knowing the routes of transmission of a contagious disease.
3. To learn about the ELISA assay, a test that uses the immune system to detect disease.

Time Allotment

One 60-minute session for exchanges and initial ELISA test. One 45-minute session to review results and perform second ELISA test on suspected carriers.

Materials

For each student:

- 2 microtubes that contain the solution representing body fluid *
- 1000 µl micropipette and tips
- waterproof marker

For each group:

- ELISA assay plate
- microtube with 1 ml positive test solution as control *
- microtube with 1 ml negative test solution as a control *
- paper towels.
- microtube with 1 ml antibody solution *
- washing solution in a flat tub large enough to fit ELISA plates *
- microtube with 1 ml color reagent solution (TMB) *
- waste beaker

Common materials:

- microtube rack for ‘non-sharing’ tubes
- chart on wall to track route of disease transmission

****Please refer to page 73 for a list a chemicals needed to make reagents***

Background Information

The immune system destroys a range of harmful organisms but respects the body's own tissues. How does it know the difference? Your cells each carry a genetically determined chemical ID. As a result, the immune system can pick off intruders such as viruses, fungi, or bacteria that carry a different chemical identification.

Special white blood cells called macrophages roam through the body. When they find an invading microbe, they engulf it and break it down into pieces. Then they display the telltale badge of the invader for other immune cells to see. The chemical badge of a foreign cell is called an antigen.

Antigens cause B-lymphocytes in the immune system to create antibodies-- molecules that lock onto the invading virus or bacterium and deactivate it. Every strain of bacterium or virus has a unique surface antigen, and the B cells use this antigen as a blueprint to make a custom designed antibody.

The ELISA (Enzyme Linked ImmunoSorbent Assay) assay detects antigen specific antibodies in a sample of body fluid. For example, the ELISA is used to detect individuals infected with the virus that causes AIDS (HIV) by screening for virus specific antibodies. The ELISA test allows someone to test for the presence of a disease agent by determining if the body has developed antibodies against the disease agent. In this test the immune system is used as a diagnostic tool.

This lab is a simulation of the ELISA test for detecting the presence of antigen from a sample of body fluid. The antigen is not real, but you should practice the same safety procedures as though it were both contagious and dangerous. If the students make errors, note that life and death decisions are made with procedures similar to this ELISA. Also, the very important point is that when you have sex (share fluids), you are exposing yourself to everyone your partner has ever been with. Below is a detailed description of the ELISA test on which this simulation is based.

The ELISA Assay

ELISAs are done in a specialized plate containing many wells.

1. A solution containing antigen is added to each well. The antigen adsorbs (sticks) onto the surface of the plate.
2. Excess or non-bound antigen in the solution is removed, and the well washed.
3. A solution containing human serum is added to each well. Specific antibodies bind to antigen, and are not removed by mild rinsing.
4. A second antibody that strongly binds to human antibody (usually made by injecting human antibodies into animals) is then added. The secondary antibody is linked to the enzyme peroxidase. If human antibody has remained in the washed well by binding to antigen, then the secondary antibody with peroxidase binds and is not removed by washing steps.
5. A solution containing a colorless peroxidase substrate (TMB) is added that the enzyme converts to a blue-green color.



Partially purified, inactivated antigens coated onto an ELISA plate



Patient serum which contains antibodies. If the patient is positive, then this serum will contain antibodies to the disease, and those antibodies will bind to the antigens on the plate.

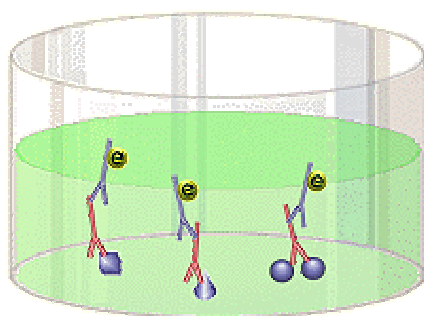


This is the second antibody, and it binds to human antibodies.

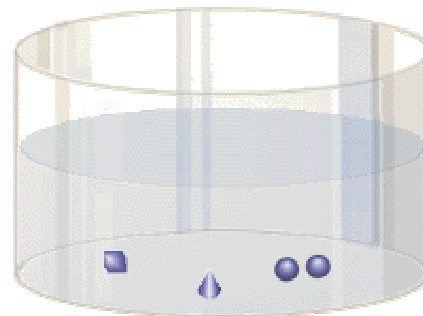


Chromogen or substrate which changes color when the cleaved enzyme attached to the second antibody, turning the color of the solution blue.

positive



negative



*Sources and Costs of Reagents

Reagent	Company	Item Number	Amount
Biotinylated Albumin	Sigma Co.	A8549	10 mg
TMB tablets	Sigma Co.	T3405	50 tablets
TMB powder	Sigma Co.	T8767	1g
Streptavidin peroxidase	Kirkegaard and Perry Co		0.5mg
Bovine Albumin	Sigma Co.	A4503	10g
Tween 20	Sigma Co.	P1379	500ml
Assay plates	Falcon Labware Co.	3911 or 3912	50 plates
Microtubes	Sarstadt Co.	72.699	1000
Sodium Chloride(NaCl)	Sigma Co	S 9888	500g
Potassium Chloride(KCl)	Sigma Co.	P 3911	500g
Disodium Phosphate($\text{Na}_2\text{HPO}_4(7 \text{ H}_2\text{O})$)	Sigma	S 9390	100g
Potassium Phosphate(KH_2PO_4)	Sigma Co.	P 0662	500g
Citric Acid	Sigma Co.	C 7129	100g
Dibasic Sodium Phosphate	Sigma	S0876	
Sodium Carbonate	Sigma	S 1641	500g
Sodium Bicarbonate	Sigma	S 6014	500g

Advance Preparation

1. Prepare the following solutions:

a) Washing Solution: 1X PBS with 0.1% Tween 20

- Make a 20X PBS stock solution. Dissolve the following in deionized or distilled water to give a final volume of 1 L:
 - 160 g NaCl
 - 4 g KCl
 - 22.4 g Na₂HPO₄
 - 4 g KH₂PO₄
- Dilute 50 ml of 20X PBS with 950 ml with water for each class.
- Add 10 ml 10% Tween 20 to this solution to make the working washing solution.

Storage Note: This buffer can be stored indefinitely at room temperature

b) Negative Solution (Sodium Carbonate): 1X Na₂CO₃ Buffer Solution

- Make a 10X sodium carbonate stock solution. Add the following to 100 ml of water:
 - 1.6 g Na₂CO₃
 - 2.9 g NaHCO₃
- Dilute this 10X stock to 1X by adding 10 ml of the stock to 90 ml deionized water. Some of this buffer will be used to make up the positive antigen solution as described below.

Storage Note: This buffer can be stored indefinitely in the refrigerator.

c) Positive Antigen Solution: Biotinylated Albumin

- Dissolve 10 mg biotinylated bovine albumin in 20 ml 1X Na₂CO₃ buffer for a final concentration of 0.5 mg/mL.
Storage Note: Store as 1 ml aliquots in the freezer.
- Prepare a 10 mg/ml bovine albumin solution by mixing 0.5 g **normal, non-biotinylated** albumin, in 50 ml deionized or distilled water.
Storage Note: The solution can be stored in 10 ml aliquots in the freezer.
- To prepare positive antigen solution. Mix the following:
 - 0.1 mL of the 0.5 mg/mL **biotinylated** bovine albumin solution
 - 9.85 mL 1X Na₂CO₃ solution
 - 50 µl of 10 mg/ml **non-biotinylated** bovine albumin.

Storage Note: The solution can be stored in for up to one week in the refrigerator.

d) Antibody Solution: Streptavidin Peroxidase

- To make the concentrate, add 1 ml of 50% glycerol solution to the 0.5 mg of streptavidin peroxidase in the purchased vial.
The concentrate is stable for years in a refrigerator.
- To make the antibody solution, mix the following:
 - 0.5 ml of 20X PBS
 - 50 µl of 10 mg/ml **normal** bovine albumin
 - 9.45 ml deionized or distilled water
 - 1 µl of glycerol/streptavidin peroxidase

Storage Note: Store in the refrigerator and use within one week after dilution.

e) Color Reagent Solution: TMB in Citrate Phosphate Solution

- Make a citrate phosphate solution (0.05 M):
1.46 g Na_2HPO_4
1.02 g citric acid
Distilled or deionized water to make a final volume of 200 ml

Storage Note: This solution may be stored indefinitely in the refrigerator

- To make the TMB solution, add:
10 ml citrate phosphate solution (see above)
1 mg TMB tablet
2 μl of 30% hydrogen peroxide

Storage Note: Use this solution on the same day and store in the refrigerator

2. Set up the microtubes that represent the student's body fluid- 2 microtubes for each student. Two students will be given 2 tubes each of the positive solution that contains the antigen that gives a positive response in the ELISA test. The remainder of the class will be given 2 tubes each of the negative solution, which contains sodium carbonate buffer only. Record which students were given the positive tubes, but **do not label the tubes positive or negative- the students should not know who has the positive solution.**
3. Label 5 microtubes 'positive' and 5 microtubes 'negative' and fill with 1 ml of the appropriate solution. Pass a set out to each group to use as controls in the ELISA assay.
4. Label 5 microtubes antibody and fill with 1 ml of the antibody solution.
5. Label 5 microtubes TMB and fill with 1 ml of the TMB solution. Keep refrigerated until ready for use.
6. Set up 5 flat tubs with enough washing solution to cover the ELISA plates. Students can reuse this washing solution.

Make sure that each student labels their tubes with their initial and '1' or '2'. The 1's will be the 'sharing' tubes, kept by the students for fluid exchange. The 2's will be the 'non-sharing' tubes, and will be put in a common rack at the front of the class. These tubes will be tested later to determine who was the initial carrier of the disease.

Teacher Notes

For the fluid exchanges, each student should work independently. For setting up the ELISA test, place the students in groups to share the assay plates. When ready for the ELISA assay, pass out a plate to each group and a set of positive and negative controls.

Following the initial ELISA test, allow the students to try and determine who the original carriers were. After the class discussion, the groups will run an ELISA test on the control tube fluids of the suspected carriers. In a typical class, from 4 to 15 students will be suspected of carrying the disease antigen. The number depends on whether you choose to eliminate some positive students or have all positive students tested. If there are fewer suspected carriers than there are groups, each suspected carrier can be tested by 2 different groups. Have the students compare results between these 2 groups to see if the results are the same.

The sample table below shows an example of the exchanges for a class of 10 students with one original carrier. The column "ELISA" represents the ELISA assay after the three exchanges. The column "Analysis" represents the class deduction of who could be the possible carrier. In this sample, either "Bill" or "Sally" must have been the original carrier. The class would have to assay the non-sharing fluids of Bill and Sally to determine which one was the original carrier. The "Bold" type shows a reconstruction of how the infection spread.

Student	Exchange I	Exchange II	Exchange III	ELISA	Analysis
Sally	Bill	Marie	Laura	pos	test
Bill	Sally	Margie	Sam	pos	test
Margie	Laura	Bill	John	pos	excluded
George	Marie	Sam	Mike	neg	
Barbara	Sam	John	Marie	pos	excluded
Mike	John	Laura	George	neg	
Laura	Margie	Mike	Sally	pos	excluded
Sam	Barbara	George	Bill	pos	excluded
Marie	George	Sally	Barbara	pos	excluded
John	Mike	Barbara	Margie	pos	excluded

Disease Epidemic – ELISA Technique

Student Pages

Introduction

In this experiment, an epidemic will be simulated, demonstrating ways in which disease organisms are spread, and the speed and ease in which disease organisms can be spread. Students will exchange ‘body fluids’ with their classmates, unaware of whom in the class is a carrier for an infectious disease. Then an ELISA assay will be used to test for the simulated disease agent in the mixed body fluids. Finally, students will become epidemiologists, researchers who study disease origin and transmission, and trace back through the transmission routes to determine the identity of the original carrier.

Materials

For each student:

- 2 microtubes that contain the solution representing your body fluid.
- 1000 µl micropipette and tips
- waterproof marker

For each group:

- ELISA assay plate
- microtube with 1 ml positive test solution
- microtube with 1 ml negative test solution.
- paper towels.
- antibody solution.
- washing solution in a flat tub large enough to fit ELISA plates.
- color reagent solution (TMB).
- A waste beaker

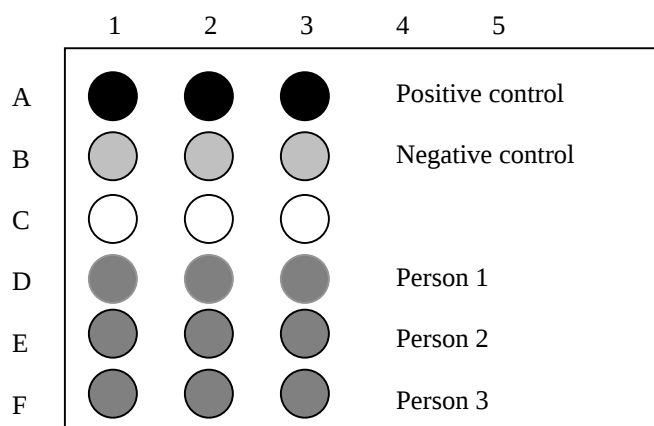
Common materials:

- Microtube rack for ‘non-sharing’ tubes
- Chart on wall to track route of disease transmission

Procedure

1. Label the two microtubes distributed by your teacher with your name. Set the non-sharing tube (marked 2) in the rack at the front of the class. Use the second identical tube or sharing tube (marked 1) for the instructions below. These tubes represent your ‘body fluid’.
2. Find one other student in your class and exchange one half of your solution from your sharing tube with him or her. Make sure that you exchange fluids at the same time.
3. Record the name of the person you first made contact with on the data sheet.
4. When everyone has completed the first exchange, find a different student to exchange one half of your sharing solution with. Record the name of your second contact on the data sheet provided.
5. When everyone has completed the second exchange, exchange one half of your sharing solution with one more student and record the name of your third contact. Be sure to choose students from all over the class, and not just in your immediate area.

6. When all three contacts have been completed you will do an ELISA test on your sharing tube fluid. Form a group with four other people. Your group will run an ELISA assay on mixtures of body fluids from each student in your group on the ELISA plate provided. Use the diagram below to help illustrate steps 7 – 9.



7. Each group will test one positive and one negative control. Add 100 μ l of a positive test solution into 3 wells and write down the well numbers on the data sheet.
8. Add 100 μ l drops of a negative test solution into a different set of three wells. Be sure to write down the well numbers on the data sheet.
9. Add 100 μ l of your sharing tube fluid into each of three wells. Record which wells contain your fluid to avoid confusing your wells with another student's.
10. After everyone has added their solution leave the plate on the lab table undisturbed for 5 minutes.
11. Shake off the fluid into a nearby sink or designated container, making sure that the fluid has emptied from each well. Tap the plate upside down onto the paper towel to remove any excess liquid or bubbles.
12. Immerse the plate in washing solution, dumping off the excess. Repeat a total of three times.
13. Remove excess solution and bubbles by again tapping the plate face down onto a paper towel.
14. Add 100 μ l of the antibody solution to each well.
15. Allow for 5 minutes incubation time on the lab table and then shake off the fluid.
16. Immerse the plate in washing solution as shown by your instructor, dumping off the excess. Repeat a total of three times (a repeat of step 12 and 13).
17. Add 100 μ l of the color reagent solution (TMB peroxidase substrate) to each well.
18. Record your observations after 5 minutes on the data sheet.

19. Along with your class, identify the negative students and positive 'infected' students on the wall chart. Try to determine who could not have been the original carrier of the disease and who are the suspected original carriers.
20. As instructed by your teacher, your group may perform a second ELISA test to analyze the non-sharing tubes carrying the body fluids of suspected original carriers.

Student Data Sheet

1. Fill in the charts below as you complete the laboratory protocol.

Your Name	Partner 1	Partner 2	Partner 3

	Samples Loaded	Results
○ ○ ○		
○ ○ ○		
○ ○ ○		
○ ○ ○		
○ ○ ○		
○ ○ ○		
○ ○ ○		
○ ○ ○		
○ ○ ○		

2. Use the attached ELISA chart to collect data from your entire class.
3. Given the collected classroom data, can you generate a list of potential original carriers?
4. Did you confirm your analysis by performing another ELISA assay on the non-shared samples? If yes, did the ELISA results support your identification of the suspected carriers?
5. What was the purpose of washing the plates in step 10-12?
6. Why was TMB added in the last step of the experiment?

7. Explain what is meant by a false positive test. Name one error that would result in a false positive test.
8. Explain what is meant by a false negative test. Name one error that would result in a false negative test.
9. What was the significance of mixing the body fluids with other students?

ELISA Data Sheet

[illegible]