Activity 1.1.5 ELISA

PLTW

ELISA

Introduction

GOALS | MATERIALS | RESOURCES

- Model how an enzyme-linked immunosorbent assay (ELISA) is used in disease detection.
- Interpret medical data and patient histories to trace disease spread
- Propose strategies for halting disease spread.

GOALS | MATERIALS | RESOURCES

- Edvotek® Mystery Infection Kit
- Micropipette and disposable tips
- Plastic transfer pipettes
- Paper towels
- Marker

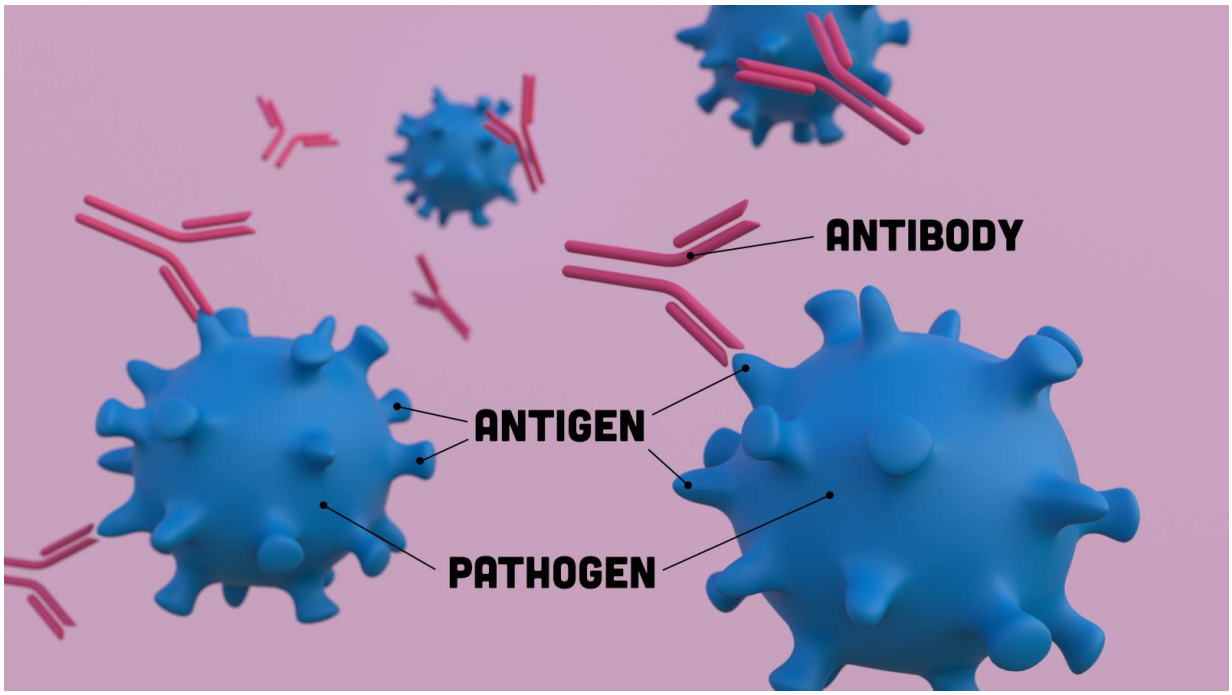
- Safety goggles
- Modeling supplies (optional)

GOALS | MATERIALS | RESOURCES

- **Activity 1.1.5 Student Resource Sheet - Serial Dilutions**
- **Care and Use of Micropipettes**
- **Activity 1.1.5 ELISA (Downloadable PDF)**

Given Sue's diagnosis, all of the patients seen in the past two days need to be called back in for immediate testing. School officials are concerned about a possible **outbreak** of bacterial meningitis on campus. To diagnose bacterial meningitis, it is necessary to obtain a sample of cerebral spinal fluid using a spinal tap. Since this procedure is extremely invasive and painful, only those patients whom doctors feel are at greatest risk for the disease will be tested. Use the spinal fluid samples to identify those who are infected with meningitis and to trace how this disease may have spread among the students on campus. Devise a plan to halt the spread of the disease before it is too late!

In PLTW Human Body Systems, you investigated the workings of the immune system and learned how *antibodies*, specific proteins produced in response to invading antigens, circulate to keep us healthy. Antibodies seek out and attach themselves to invaders, flagging them for destruction by the immune system. These antigens are molecules foreign to the body and can include bacteria, viruses, and fungi. Because antibodies are extremely specific to the antigens they attack, these proteins can be used in the laboratory to help identify disease agents.



One test, the **enzyme-linked immunosorbent assay (ELISA)**, combines targeted tagging with antibodies and an **enzyme** reaction that produces a visible color change, to test for the presence of disease antigens or antibodies produced in response to that antigen. The ELISA can even detect disease agents in body fluids before the body has a chance to mount an immune response and produce antibodies. An ELISA can provide *qualitative results*, indicating whether a patient is positive or

negative for the presence of the **antigen** or **antibody**, or an ELISA can provide *quantitative results*, determining how much of the detected substance is present.

In this lab, you will use ELISA to test simulated cerebral spinal fluid (CSF) samples taken from patients at Sue's school for the presence of bacterial meningitis. This rapid test can be completed in less than one hour and can detect antigens of the *Neisseria meningitidis* bacteria. Your job is to determine which college students are infected with this deadly bacterium and to propose a strategy for halting further spread. For those who are infected, use quantitative data to propose a chain of infection. Use data from the ELISA test and from the patient histories to trace how this disease may have spread from person to person.

Part I: Principles of the ELISA Test

1. Use reliable sources to review the relationship between antigens and antibodies.

2. In your laboratory notebook, write a paragraph that explains how antibodies work to keep us well. Underline each of the following terms in your paragraph:

   - Antigen
   - Antibody
   - Immune system
   - B lymphocytes

3. As you watch the ELISA Animation video, consider how scientists can use antibodies to help detect disease antigens. Use the following key to assist you in understanding the ELISA reaction.

   - Proteins
   - Primary Antibody
   - Enzyme-Linked Secondary Antibody

- Enzyme Substrate

ELISA Animation video (The video has no sound.)

4

Use what you learned in the video to draw a labeled and colored sequential diagram or to construct a small interactive model that shows how the ELISA test can be used to identify patients who are infected with bacterial meningitis. Draw your diagram or sketch your model in your laboratory notebook. Your drawing or model should include the following labeled components:

- Antigen
- Primary antibody
- Secondary antibody

- Enzyme
- **Substrate**
- *Neisseria meningitidis* bacteria

5 Share your drawings or model with the class.

6 Address the following prompts in your lab notebook.

Laboratory Notebook

- Explain why antibodies allow scientists to target and identify specific disease agents.
- Why is the secondary antibody used in an ELISA test conjugated with an enzyme? What happens when this enzyme meets up with its substrate?

Part II: Serial Dilutions

7. Obtain an **Activity 1.1.5 Student Resource Sheet - Serial Dilutions**.

8. Read the information presented about Serial Dilutions.

9. Use information from the resource sheet to complete practice problems A through C found at the bottom of the resource sheet.

10. Compare your answers with a partner. Correct any mistakes.

11. Answer the reflection question below and then compare your answer with a classmate.

Reflection

Disease samples from two patients were collected and subjected to serial dilutions before running an ELISA. What does it mean if the disease can be detected in samples from one person only at a dilution of 1/5, but the disease can be detected in the other patient at a dilution of 1/5 and 1/100?

Part III: Running an ELISA

Use the ELISA test to determine which patients test positive for the presence of the disease antigen and to determine the relative concentration of the bacteria in the fluid of those who are affected. Early findings assure adequate treatment to those who are affected and provide information necessary to prevent the disease from spreading all over the college.

In this lab activity, you will test the simulated CSF of two of the patients presented in Activity 1.1.2 and Activity 1.1.3. These samples have randomly been assigned by your teacher.

12. Review the **Care and Use of Micropipettes** resource as they require great care when being used.
13. Put on your safety goggles.
14. Obtain a copy of the laboratory protocol from your teacher.
15. Carefully read this entire protocol. Note that your teacher will provide guidance on when to complete

each step of the experiment.

16 Locate two 12-well strips at your lab station. Place one strip off to the side. You will use one strip to prepare your concentration standards and one strip to complete your ELISA test.

17 Use a marker to label the outside wall of each well on one 12-well strip with the numbers 1 through 12 (Figure 1). This strip will serve as your concentration standards.

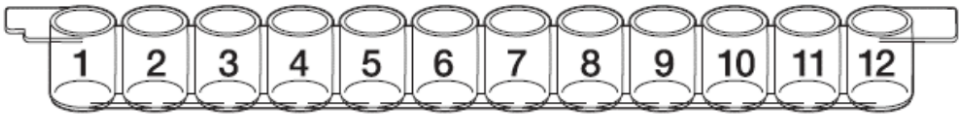


Figure 1. Numbered Micro Wells

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18 Prepare your standard strip as directed by your protocol.

19 Calculate the dilution in each well. Remember that the antigen is provided at a concentration of 100 µg/mL. Think about what you have added to each well and about the practice **serial dilution** questions you completed in Part II, step 9.

20 Record your dilutions in Table A on the laboratory protocol. If your teacher directs you to, attach all data tables from this experiment in your laboratory noteboook.

21 Carefully place the standard strip off to one side.

22 Obtain the second 12-well strip. This strip will function as your test strip. Label the strip with the numbers 13 through 24.

- Wells 13 through 15 will serve as positive controls.
- Wells 16 through 18 will serve as negative controls.
- Wells 19 through 21 will be used to test the first patient you have been assigned.
- Wells 22 through 24 will be used for the second patient you have been assigned.

23 Record the names of the patients you will be testing in Table B of the protocol.

24 Follow the directions in the protocol to load your control and patient samples, wash the wells, add the antibodies, and add the substrate.

25 When you have completed all steps, observe the color of the reactions in your positive and negative samples to confirm that the ELISA has succeeded.

26 Determine an initial positive or negative diagnosis for the patients you are testing and record your findings in Table B.

27 Using your concentration standards, approximate the level of infection for any patient that tests positive in Table B. Refer to Table A for the concentration values in each well of the standard strip.

28 Share your findings with other lab groups.

29 In Table C of the protocol, record results for all nine patients who are being tested. For those patients who tested positive, use the concentration data as well as the patient history information presented in Activity 1.1.2 Investigating an Outbreak and Activity 1.1.3 Using DNA to Identify Pathogens to deduce a possible path of infection. Lower levels of disease antigen may signal a newer infection. Refer to the connections chart or web you started in Activity 1.1.2 Investigating an Outbreak.

30 In your laboratory notebook, explain who you believe was the first person infected and describe how the infection might have spread from this person to the rest of those with the disease. Provide evidence for your theory.

31 Share your theory with the class.

32 Explore the Bacterial Meningitis tour models. There are two chapters. Be sure to interact with the images and read the content in each chapter.

Open the **Bacterial Meningitis** tour of models in a new browser tab.

33 In your laboratory notebook, describe how patients are physically affected by bacterial meningitis and explain why this is such a serious infection. Explain how the

name of the disease relates to the anatomy of the brain. Complete additional research as needed.

34 Research appropriate treatment for bacterial meningitis and describe a comprehensive plan to treat those affected as well as prevent the spread of the disease on campus. Describe your plan in your laboratory notebook.

35 Share your ideas with the class and come to class consensus on how to respond to the outbreak.

Conclusion

Question 1

Describe a situation that illustrates why it is a good idea to complete the ELISA in triplicate.

Question 2

Why do you think college students living in dorms are populations that often see meningitis outbreaks?

Question 3

How did ELISA data allow you to track the path of infection at the college?

Question 4

Discuss the limitations of using antigen concentration to deduce the path of infection. Be sure to refer to the workings of the human immune system.

Question 5

The ELISA test can also be used to detect antibodies that are produced in response to a specific antigen. Using information about how you completed this ELISA experiment, outline a procedure to test for antibodies in the blood.

Question 6

Explain why in sudden outbreaks, it may be better to test for disease antigens than for antibodies.

Question 7

Home pregnancy tests use ELISA technology. When a woman is pregnant, her body produces a hormone called human chorionic gonadotropin (hCG). Explain how antibodies can be used to detect this hormone and are linked to the color change a woman may see on a positive test.