Name _____ Class _____ Date_____

Detecting Disease

Performing a Lyme Disease Assay

Question How can you tell if a person is infected with the bacteria that cause Lyme disease?

Lab Overview You will take on the role of a medical laboratory technician in a diagnostic lab and test simulated blood serum samples using a test called an Enzyme-Linked Immunosorbent Assay (ELISA).

Introduction Students from Ms. Garcia's biology class went on a field trip to study plant communities in the hills near their school. After the trip was over, one student noticed a tick on her leg. The tick was identified as a black-legged (deer) tick. This tick species is often host to *Borrelia burgdorferi*, the bacteria that cause Lyme disease. Other students developed possible Lyme disease symptoms. For example, one student developed an unusual skin rash with a large red spot that grew bigger each day. Another student developed fever and muscle aches.

You will take on the role of a medical laboratory technician in a diagnostic lab. You will test three samples of simulated blood serum (plasma without blood-clotting proteins) from students in Ms. Garcia's class using a procedure called an ELISA test. This test is similar to those used in real medical diagnostic labs.

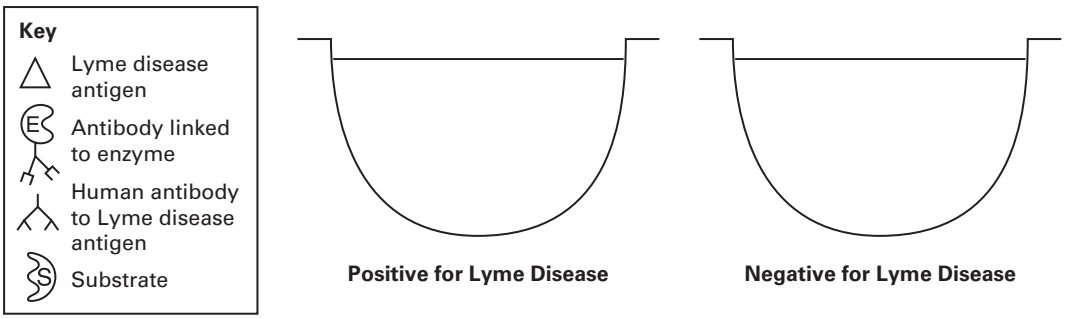
Background The ELISA test is based on the specific fit of an antibody to only one type of disease antigen. For example, antibodies that "match" the Lyme disease antigen will bind tightly to that type of antigen only. Antibodies that "match" other antigens will not bind at all to Lyme disease antigens.

In the model ELISA test you will first add simulated Lyme disease antigen, which consists of proteins, to a set of wells on a plastic plate. The protein molecules will bind to the plastic wells. Next, you will add to the wells simulated blood serum samples from patients who are being tested for Lyme disease. If a patient has been exposed to *B. burgdorferi*, the patient's blood serum should contain the antibody to the Lyme disease antigen (this antibody is referred to as the primary antibody). If present, the primary antibody will bind to the Lyme disease antigen molecules that are stuck to the plastic wells.

To some other wells, you will add a positive control solution known to contain the antibody to the Lyme disease antigen. To the last set of wells, you will add a negative control solution known *not* to contain the antibody to the Lyme disease antigen. The control solutions will help you confirm the results of your patient sample tests.

After adding the patient samples, positive control solution, and negative control solution, you will rinse the wells. Any antibodies or other proteins not bound to the Lyme disease antigen will wash away. Next, you will add a solution containing another antibody bound to an enzyme. This other antibody (called the secondary antibody) will bind to the primary antibody if it is present. Then you will rinse the wells again. In the final step you will add a solution containing a color-producing chemical (called the substrate). If the secondary antibody with the enzyme is still in the wells, then the enzyme will act on the substrate. A colored product will form, giving the liquid in the well a purple-pink color. If the secondary antibody and enzyme are not present, no color change will occur.

Prelab Activity Use the symbols in the key below to draw a sketch showing the contents of a positive and negative well after all the steps of an ELISA test have been completed. Your sketch should indicate the reactions that occur among the substances. Afterward, answer the Prelab Questions.



Prelab Questions

1. Describe the contents of each well in your sketches. What do the two wells have in common?

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2. If antibodies bind to the Lyme disease antigen in the ELISA plate well, how do you know that the antibodies were released in response to a *B. burgdorferi* infection?

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3. Would either the negative or positive control solution, or both, contain the primary antibody? Explain.

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4. Suppose an ELISA test of a patient who had been exposed to *B. burgdorferi* several weeks before produced a negative result. Which of the following could be a possible explanation? Explain your response.
   a. There was no primary antibody in the serum.
   b. You did not change pipettes between samples.
   c. You didn't allow enough time for the antigen to bind to the well.

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5. When performing ELISA tests in a medical diagnostic lab, it is important to change pipettes between patient samples. Why?

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Materials

- ELISA multi-welled plate
- marker
- 3 simulated blood serum samples
- positive control solution
- negative control solution
- disposable transfer pipettes or micropipettor and tips
- Standard Lyme Disease Antigen solution
- antibody-linked enzyme solution
- color-producing substrate solution
- wash buffer in wash bottle
- paper towels
- clock or watch

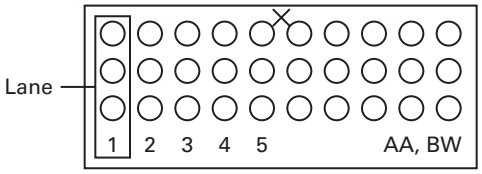
Procedure

Part A: Setting up a Sample Key

1. To ensure that patients receive proper care, it is vital to keep accurate records. In the space below record the identification numbers marked on your three simulated blood serum samples.

   Identification numbers:

2. You will test each patient sample and control solution three times, so you will need three wells for each sample. As shown in the sample ELISA plate below, each set of three wells is called a "lane." The lanes are numbered from left to right. Prepare a key to record the lane you will use for each sample.



Sample ELISA plate

Key for Investigation

Sample	Lane

3. Use a marker to write an X on the ELISA plate showing where your lanes end (see the sample above). You may also want to label the plate with your group's initials.

Part B: Performing the ELISA Test

1. Load the Lyme disease antigen solution into all 15 wells. Let the antigen sit at room temperature for the amount of time specified by your teacher. This will give the antigen adequate time to bind to the wells.
2. Use wash solution to rinse the wells, removing any antigen that has not bound to the wells.
3. Using a new transfer pipette for each sample, load the simulated blood serum samples, positive control solution, and negative control solution into the appropriate wells. Let the plate sit for the amount of time specified by your teacher.
4. Rinse the wells again with wash solution to remove any unbound antibodies and other proteins.
5. Load the secondary antibody-enzyme solution into all 15 wells. Let the plate sit for the amount of time specified by your teacher. Rinse the wells to remove any unbound antibody-enzyme solution.

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6. Add the color-producing substrate to all 15 wells. Watch for the liquid in any of the wells to turn purple-pink. This color change indicates a positive result, meaning antibodies to the Lyme disease antigen are present in the sample. Record your results in Data Table 1 by placing a check mark in the appropriate column.

Data Table 1

Sample		Positive for Antibody	Negative for Antibody
Patient 1	Sample 1		
	Sample 2		
	Sample 3		
Patient 2	Sample 1		
	Sample 2		
	Sample 3		
Patient 3	Sample 1		
	Sample 2		
	Sample 3		
Positive control	Sample 1		
	Sample 2		
	Sample 3		
Negative control	Sample 1		
	Sample 2		
	Sample 3		

Analysis and Conclusions

1. Did any of your patient samples test positive for antibodies to the bacteria that cause Lyme disease? Explain.

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2. Summarize the reactions that lead to a positive ELISA test for Lyme disease.

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3. Sometimes medical labs have different technicians test samples from the same patient to reduce the possibility of technician error. Compare data with your classmates to determine whether they observed the same results as you did for patients 1, 2, and 3. Summarize your findings and suggest possible reasons for any differences you note.

4. Explain the purpose of making a key for the samples on the ELISA plate.

5. Explain the purpose of having three of each sample on the plate.

6. What might have happened if you didn't wash the plate after adding the secondary antibody?

Extension

Research how Lyme disease is treated and how it can be prevented. Then create a public awareness poster or public service announcement for radio or television that describes your findings. Consider ways to make your poster or announcement capture people's attention and deliver useful information.