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EDVOTEK AT-HOME:

ELISA

Experiment Objective:

In this experiment, students will master the experimental concepts and methodology involved with a quantitative enzyme-linked immunosorbent assay (ELISA).

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Instructions

1. Carefully **READ** the background.

2. **HIGHLIGHT** any words or phrases that you don't know. **LOOK UP** the definition, and **WRITE** the definition into the margins.

3. **UNDERLINE** important information about the experiment.

4. Use this information to **FILL IN** the role of each experimental component.

5. **READ** through the experiment, pausing to **ANSWER QUESTIONS** about what is happening.

6. **ANALYZE** the results.

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Background Information

Antibodies (also called immunoglobulins, or Igs) are specialized proteins that allow the immune system to distinguish between "self" and "non-self" proteins or polysaccharides. These Y-shaped molecules comprise four linked polypeptide chains: two identical "heavy chains" and two identical "light chains" (Figure 1). The antigen binding sites are located at the ends of the short arms of the Y. The amino acid sequence region is variable, allowing for each antibody to recognize a unique **epitope** (a particular location within an antigen).

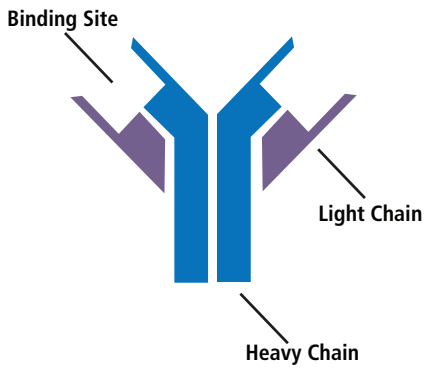

Figure 1: Structure of an antibody

Antibodies used in scientific research are produced as an immune response when animals (i.e. rabbits, mice and guinea pigs) are injected with an antigen. The immune response will produce antibodies that are specific to the antigen, which are then purified from the serum. This solution will contain a mixture of antibodies because different immune cells will create antibodies that recognize different epitopes of the antigen. This heterogeneous mixture of antibodies is called a **polyclonal antibody**. If we isolate and culture individual immune cells from these animals, we can create **monoclonal antibodies**. These antibodies are directed against a single epitope, and thus are very specific. Because of their specificity, monoclonal antibodies can be used to detect the presence of specific biomolecules (i.e. peptides, proteins, antigens and hormones) in a complex sample. To quantitatively detect the presence of molecules within a sample, scientists use the Enzyme Linked Immunosorbent Assay (ELISA). These samples can be single proteins or complex mixtures like cellular lysates. The ELISA is commonly used for medical diagnostics, as it can be used to identify antigens in blood, urine, spinal fluid, and other biological samples. An ELISA can be designed to provide qualitative or quantitative results. In a qualitative ELISA, the results will indicate if a sample is positive or negative for the antigen. This type of assay is simple to perform and is useful for situations where the exact concentration of molecules is not necessary, such as pregnancy or drug tests. Alternatively, quantitative ELISAs use a standard curve to determine the precise concentration of a substance in the sample.

The traditional ELISA requires two antibodies. One antibody, called the **primary**, recognizes the antigen of interest. For example, an ELISA that detects the HIV virus would use an antibody that recognizes one of the virion's coat proteins. The **secondary** antibody recognizes the primary antibody – if a rabbit produced our primary antibody, we would use a secondary antibody that recognizes rabbit antibodies. The secondary antibody is covalently linked to an enzyme called Horseradish Peroxidase (HRP) that lets us detect the presence of the antibody-antigen complex (Figure 2A). HRP has a high catalytic activity – its substrate turnover rates exceed 10^6 per second – allowing us to quickly detect even the smallest amount of antigen.

To perform an ELISA, the samples are added to the wells and the antigens are allowed to adsorb to the surface through hydrophobic associations (Figure 2B). ELISAs are performed in transparent plastic microtiter plates, which allow scientists to easily visualize the results. These plates contain many small wells into which the samples are deposited and analyzed.

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Background Information, continued

In this experiment, positive samples will contain simulated antigens that can stick to the wells. After briefly washing the wells to remove unbound sample, the primary antibody is added to each well. If the antigen is present, the primary antibody will bind to it and remain attached after washing. Next, a secondary antibody is added and will only adhere where primary antibody has already bound. Finally, the substrate ABTS is added to each well - if the secondary antibody is present the HRP enzyme will catalyze a reaction that produces a colored product from the ABTS. The data collected will allow students to diagnose patients as positive or negative, and to quantify the levels of bacterial antigen in the positive patients.

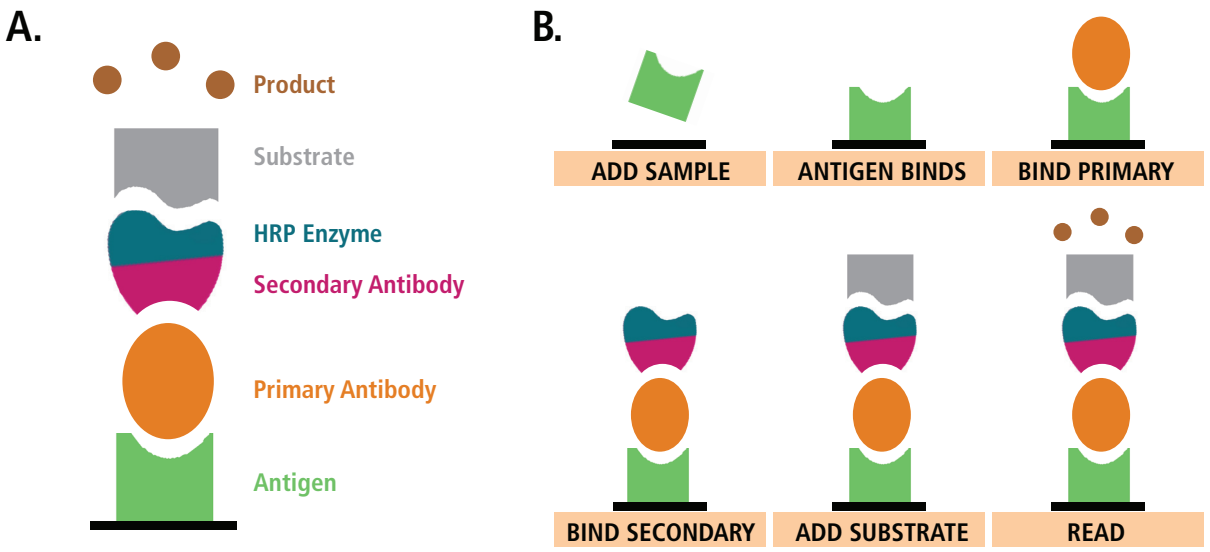

Figure 2: Optimized ELISA workflow

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Experiment Components

ACTIVITY 1

Fill in the purpose of each experimental component.

Component	Purpose
• Wash Buffer	_____
• Antigen	_____
• Primary Antibody	_____
• Secondary Antibody	_____
• ABTS	_____
• Small transfer pipets	_____
• Strip tubes (12 well)	_____
• Disposable lab gloves	_____
• Safety goggles	_____
• Adjustable volume micropipettes and tips	_____

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Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will master the experimental concepts and methodology involved with a quantitative enzyme-linked immunosorbent assay (ELISA).

OVERVIEW

The ELISA will utilize 12-well plastic strips – the first strip will be used to create a standard curve, while the second strip will allow you to quantify the amount of antigen in control and patent samples.

The standard curve will be created using a 100 µg/mL solution of antigen and dilution buffer. At every dilution you will cut the concentration of antigen in half, resulting in a wide range of protein concentrations across the dilution series. This standard curve will then be compared to control and patient samples to determine who is expressing the antigen.

During the experiment you will use an adjustable volume pipette to add the different components to the plastic strips. It is important to use good, accurate pipetting techniques to ensure the correct amount is added to each well. In addition, replacing the tip between components and using care to prevent overflowing the wells during the washes will help to prevent cross-contamination.

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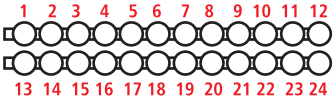
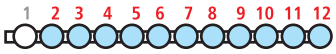
Module I: Performing a Quantitative ELISA

PREPARATION OF STANDARDS

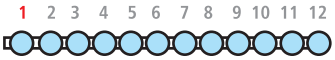


1. **OBTAIN** two 12-well strip tubes. **LABEL** one strip with numbers 1-12 and the other with numbers 13-24. Set strip 13-24 off to the side for now.

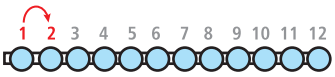
2. Use a micropipette to **ADD** 50 µL Dilution Buffer to wells 2-12.



3. **ADD** 100 µL of Antigen to well #1. (The antigen is provided at a concentration of 100 µg/mL.)



4. **PIPETTE** 50 µL from well #1 into well #2.

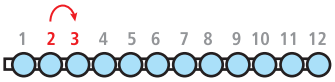


Q: What is the new volume of well 2?

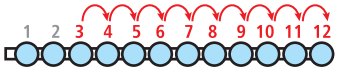
Q: What is the concentration of antigen in well 2?

5. Fully **MIX** the sample by gently pipetting up and down 5 times.

6. Using the same pipette tip, **TRANSFER** 50 µL from well #2 into well #3. **MIX** as in step 5.



7. Continue to serially **DILUTE** the remaining samples through well #12.



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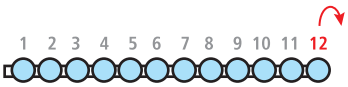
Module I: Performing a Quantitative ELISA, continued



8. **REMOVE** and **DISCARD** 50 µL of the diluted antigen from well #12.
   Q: Why remove 50 µL from well #12?

9. Set the strip off to the side where it will not be disturbed. **FILL** in the dilutions and antigen concentrations for each well in Table A.

Table A: Dilutions and Concentrations						
Well #	**1**	**2**	**3**	**4**	**5**	**6**
Dilution	---					
Concentration	100 µg/mL					
Well #	**7**	**8**	**9**	**10**	**11**	**12**
Dilution						
Concentration						

Q: What is a serial dilution?

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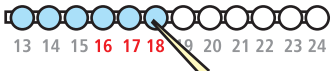
Module I: Performing a Quantitative ELISA, continued

LOADING THE CONTROL AND PATIENT SAMPLES

10. **RETRIEVE** the Control and Patient Samples.

11. Using a micropipette, **ADD** 50 µL of Positive Control sample to wells 13-15. **REPLACE** the pipette tip.

12. **ADD** 50 µL of Negative Control sample to wells 16-18. **REPLACE** the pipette tip.



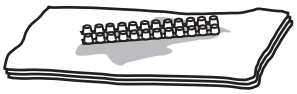
13. **ADD** 50 µL of your first Patient Sample to wells 19-21. **REPLACE** the pipette tip.

14. **ADD** 50 µL of your second Patient Sample to wells 22-24.

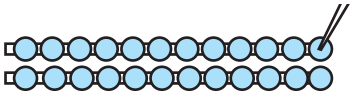
15. **RETRIEVE** the Standard Curve strip from Step 9. **INCUBATE** both strips for 5 min. at room temp.
    Q: What is happening in the well during this incubation?

REMOVAL OF SAMPLE AND WASHING THE WELLS

16. **INVERT** the strips over the sink or a stack of paper towels to remove the samples. Gently **TAP** the strips 4-5 times onto a fresh paper towel. **DISCARD** the wet paper towels.

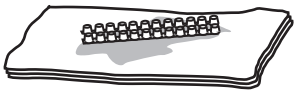


17. Using a transfer pipet, **ADD** Wash Buffer to fill each well, being careful not to overfill. *NOTE: To minimize cross-contamination it is important that you avoid spilling buffer into neighboring wells.*



18. **REPEAT** step 16 to **REMOVE** the wash buffer.

19. Using the same transfer pipet, **REPEAT** the wash a second time. **INVERT** the strips onto fresh paper towels and **TAP**.
    Q: What is being washed out of the wells?



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Module I: Performing a Quantitative ELISA, continued

ADDITION OF PRIMARY AND SECONDARY ANTIBODIES

20. Using a new micropipette tip, **ADD** 50 µL of Primary Antibody to each well.
    Q: What is the role of the Primary Antibody?

21. **INCUBATE** for 5 minutes at room temperature.
    Q: What is happening in the well during this incubation?

22. **INVERT** onto paper towels and **TAP. WASH** the wells twice as in steps 16-19.
    Q: What is being washed out of the well?

23. Using a new micropipette tip, **ADD** 50 µL of the Secondary Antibody to each well.
    Q: What is the role of the Secondary Antibody?

24. **INCUBATE** for 5 minutes at room temperature.
    Q: What is happening in the well during this incubation?

25. **INVERT** onto paper towels and **TAP. WASH** the wells twice as in steps 16-19.
    Q: What is being washed out of the well?

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Module I: Performing a Quantitative ELISA, continued

ADDITION OF SUBSTRATE

26. Using a new micropipette tip, **ADD** 50 µL of ABTS to each well.
    Q: What is the role of the ABTS?

27. **INCUBATE** the plate for 2-5 minutes at room temperature, or until color no longer changes in the wells with the highest antigen concentrations.
    Q: What is happening in the well during this incubation?

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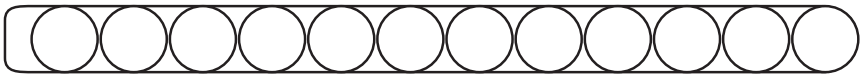
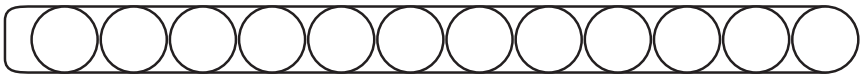
Student Worksheet

PATIENT IDENTIFICATION

Patient 1: _____ **Patient 2:** _____

Strip Tube Templates:





1. **LABEL** the trip tube. ABTS creates a blue color when it catalyzes its reaction. **COLOR** in each well according to what you hypothesize it will be.

2. **EXPLAIN** the steps of an ELISA in your own words.

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Module II: Analysis of Quantitative ELISA Results

1. **OBSERVE** the color of the reactions in your positive and negative samples to confirm that the ELISA has succeeded. **DETERMINE** an initial positive or negative diagnosis for the patient samples and **RECORD** it below or in your lab notebook.

REFER TO INSTRUCTORS FOR IMAGES OF RESULTS.

2. Using the standard strip, **ESTIMATE** the concentration of your patient samples and **RECORD** the results in Table B, below.

Table B: Identification of Patient Samples						
Well #	**13**	**14**	**15**	**16**	**17**	**18**
Sample Name	Positive Control			Negative Control		
Concentration						
Well #	**19**	**20**	**21**	**22**	**23**	**24**
Sample Name						
Concentration						
Diagnosis						

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