Determining Disease Exposure Using ELISA

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Table of Contents

Science Background

ELISA

An ELISA, or an enzyme-linked immunosorbent assay, is a laboratory tool that allows us to visualize the presence or absence of an antibody or antigen. Because we know that antibodies will bind to specific antigens, we can test for the presence of an antigen by exposing a substance to antibodies and determining whether or not binding has occurred. The most powerful and sensitive type of an ELISA is called a sandwich ELISA. In a sandwich ELISA, a polystyrene microplate is coated with a primary antibody. Sample is then added which may or may not contain antigen that will bind to the primary antibody. A secondary antibody is then added which will bind to the antigen, if present. The secondary antibody is conjugated with an enzyme that will change color in the presence of a substrate. In the last step, the substrate is added to the wells. By using antibodies that have been conjugated with enzymes that change color in the presence of an added substrate, a color change allows us to determine when an antibody has bound to an antigen. An ELISA can also be quantitative with the degree of color change correlating to the concentration of the measured substance. This laboratory activity will enhance students understanding of modern laboratory techniques. It demonstrates the fact that in biology, we often use proxy measurements, because we are testing for things that we cannot actually see. In the case of an ELISA, we will be able to see a color change that occurs due to a chemical reaction which happens only if an antigen is present in the sample and binds to an antibody. What we see is the color change. What it tells us is the presence of an antigen. Depending on the placement of this lab activity throughout the course of the year, instructors could refer back to other Advanced Placement (AP) labs that rely on proxy measurements. For example, the speed at which a leaf disc floats tells us the rate of oxygen bubble production and thus the rate of photosynthesis.

Understanding the specificity of antigen/antibody interactions is crucial to understanding how an ELISA works. This unit uses the ELISA technique as a real-world application through which to teach students the basics of immunology.

Immune System

The difference between innate and adaptive immunity is that innate immunity is a generalized response to any pathogen, while the adaptive immunity is a specific response to one specific type of pathogen. It is essentially the difference between the immune system recognizing a generic pathogen to influenza A H1N1. Innate immunity first consists of barriers that prevent pathogens from entering the body, such as skin, mucous secretions, sweat, tears, and stomach acid. Innate immunity also consists of cells that specifically engulf and destroy pathogens. Such cells include macrophages, dendritic cells, and neutrophils.

In adaptive immunity, B cells and T cells each have unique receptors that can bind with a specific antigen. Antigens are unique proteins present on the surface of pathogens. After a cell of the innate immune system engulfs a pathogen, it will present the antigen on the surface. A B cell or T cell with a matching antigen receptor with bind with the antigen. This will then activate the B cell and T cell, triggering them to divide to make more cells that will recognize the specific antigen and also triggering them to release chemicals to signal other cells of the immune system.

As B cells divide to make more B cells that will bind with the specific antigen, a specific subset of B cells called plasma cells secrete antibodies. Antibodies are proteins that bind to a specific antigen. The antibodies will bind to the pathogen, disabling it and marking it for destruction.

The specificity of binding between an antigen and an antigen receptor, and in turn an antibody, is the key to the adaptive immune system. The specific binding occurs due to a matching shape and intermolecular forces between the antigen and the receptor. This relationship between the structure of the molecules and their functions is a major repeating theme in biology. Students may have been previously exposed to the concept of binding affinity through enzyme/substrate interactions

References

"Overview of ELISA | Thermo Fisher Scientific - US." *Thermo Fisher Scientific*, Thermo Fisher Scientific, www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html.

Urry, Lisa A, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, Rob Jackson, and Jane B. Reece. "Immune System." *Campbell Biology in Focus*, 2014. Print.

Student Outcomes

Science concepts covered in this unit: innate vs adaptive immunity, roles of specialized cells in the immune system, binding specificity, ELISA protocol. The immune system is relevant to the broader concepts of homeostasis and cell communication that are emphasized in the AP Biology curriculum. Emerging diseases and pandemics are relevant to students lives, because everyone is susceptible to infectious disease, and both personal health decisions and public health policies affect the spread of disease.

- Students will read and discuss current event news stories related to emerging diseases and pandemics.
- Students will learn the difference between innate and adaptive immunity through direct instruction and readings.
- Students will design a model to demonstrate the principles behind an ELISA protocol.
- Students will learn to use micropipettes and perform an ELISA protocol.
- Students will interpret the results of an ELISA to answer a scientific question.

NGSS Standard Alignment:

- Disciplinary Core Ideas: LS1.A:
  - Systems of specialized cells within organisms help them perform the essential functions of life.
  - Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level.
  - Feedback mechanisms maintain a living system's internal conditions within certain limited and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (positive feedback) or discourse (negative feedback) what is going on inside the living system.
- Crosscutting Concepts:
  - Systems and Models: Models can be used to simulate systems and interactions within and between systems at different scales.
  - Structure and Function: Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, and structure of different components, and connections of components to reveal its function and/or solve a problem.
  - Stability and Change: Feedback (negative or positive) can stabilize or destabilize a system.
- Science and Engineering Practices:
  - Developing and Using models: Develop and use a model based one evidence to illustrate the relationship between systems or between components of a system.
  - Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data and refine the design accordingly.
  - Construct an explanation based on valid and reliable evidence obtained from a variety of sources and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.

AP Biology standard alignment:

- Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.
- Essential knowledge 3.D.2: Cells communicate with each other through direct contact with other cells or from a distance via chemical signaling.
- Essential knowledge 3.D.3: Signal transduction pathways link signal reception with cellular response.
- Essential knowledge 3.D.4: Changes in signal transduction pathways can alter cellular response.

Placement in AP Biology course

This unit will fit best in the context of homeostasis and or cell communication. Because the concepts in this unit deal with specialized cells and membrane-bound receptors, this unit should be taught after students have learned about cell structures and cell membranes.

Learning Objectives

- Students will be able to describe the difference between innate and adaptive immunity.
- Students will correctly differentiate between the roles of various specialized cells within the immune system.
- Students will work collaboratively to accurately model an ELISA protocol.
- Students will successfully work in groups to perform an ELISA protocol.
- Students will correctly interpret the results of their ELISA protocol to determine exposure to a pathogen.
- Students will make connections between diagnostic tools and public health decisions.

Time Requirements

6 50-minute periods

Advance Preparation

Instructors should plan to order an ELISA kit 3 weeks in advance of completing the lab, in case there is a delay with availability or shipping.
Preparing the student stations and reagents from the ELISA kit will take approximately 1 hour.

Materials and Equipment

Direct instruction on immune system and ELISA protocol will require:

- computer
- projector
- paper copies of news articles or student access to internet

Modeling the ELISA protocol prelab activity will require: a variety of materials for students to manipulate to create their own model.

- Playdough
- modeling clay
- construction paper

- pipe cleaners
- paperclips
- clothespins, etc.

ELISA protocol will require:

- ELISA kit should include the following:
  - Antigen
  - Primary antibody
  - Secondary antibody
  - HRP enzyme substrate
  - 10% PBS (phosphate buffered saline)
  - 10% Tween 20
  - Disposable plastic transfer pipets
  - Bottles and caps
  - Microplates
  - 2.0 ml micro test tubes
  - http://www.bio-rad.com/en-us/product/elisa-immuno-explorer-kit?ID=1e3f3100-99f6-49b3-b9a0-2c8aad9d9285
- Adjustable micropipettes 20-200 microliters OR fix volume pipette of 50 microliters
- Paper towels
- Permanent markers
- Pipet tips
- Beakers
- Graduated cylinders: 100 ml and 1 L
- Distilled water

Estimated costs:
ELISA kit from Biorad is $138
Adjustable micropipettes are a minimum of $60 each, boxes of tips are around $10
Materials for modeling will vary in cost or could be provided by students.

Possible sources for access to expensive equipment
Local labs or universities or area education associations may have programs for loaning out micropipettes.

Precautions and safety
Students should wear goggles and gloves for the duration of the lab and sterile technique should be demonstrated. New pipette tips should be used when switching solutions. Emphasize the need to control for cross contamination.

Student Prior Knowledge and Skills

Expected prior content knowledge
Students in an AP Biology class should have basic background knowledge in cell structures and organelles. Before starting this unit, students should have completed the unit on cell membranes, so they should also understand membrane bound receptors. This unit is meant to be part of the unit on cell communication and homeostasis, so students should have a developing understanding of feedback mechanisms, types of cell communication, and signal transduction.

Expected prior technical skills
Students in an AP biology course should have already completed one year of introductory biology and one year of chemistry. Metric measurements, dilutions, and error analysis are skills they should have mastered.

Possible preconceptions
The most probable preconception a student may have regarding the immune system is a misunderstanding of vaccines and immunity. The internet makes unreliable information very easy to find and disseminate, so some students may arrive with misconceptions about the dangers and effectiveness of vaccination. Instructors should be aware of this and make sure to present objective information.

Daily Unit Plans

Day 1
- Discussion on the following articles (20 min)
  - https://harvardmagazine.com/2007/03/the-sars-scare.html
  - https://www.newsweek.com/new-sars-virus-discovered-myanmar-bats-1020810
  - https://www.businessinsider.com/pandemic-virus-simulation-johns-hopkins-shows-vulnerability-2018-7
  - The first article is long, so it should be edited for length at the instructor's discretion.
  - Assign articles for homework to save class time.
  - Students should be divided into three groups. Each group will be responsible for reading one article and presenting the main points to the class.
    - Students will analyze the article using a "CER" chart, documenting the main claims, supporting evidence, and reasoning for each claim.
    - Each presenting group will guide the remainder of the class through the CER chart for the article being presented.
    - At the end of the three presentations, each student will have a CER chart for each article.
    - See appendix for sample CER chart handout.
- Possible discussion questions:
  - What types of transmission would make a disease more difficult to contain?
  - What are some challenges in diagnosing an emerging disease?
  - What are challenges are involved in instituting policies that could help contain a new disease?
- Lecture presentation on innate vs adaptive, cells of immune system (30 min)
- Homework: HHMI Click and Learn on cells of immune system - http://media.hhmi.org/biointeractive/click/immunology_primer/01.html

Day 2:
- Students work in small groups to develop an analogy for the different cells in the adaptive and innate immune system.
  - Many teachers will be familiar with introductory biology lessons in which students create an analogy for cell organelles, for example, comparing each organelle to the part of a city or factory. A similar approach should be used for this activity. (20-25 min)
- Formative assessment: students work in pairs to create a graphic organizer of steps of immune response to a pathogen. (25 min)
  - See appendix for example.
- HW: textbook reading to reinforce concepts

Day 3:
- Direct instruction on ELISA (20 min)
  - Optional video to aid description https://www.youtube.com/watch?v=RRbuz3VQ100
- Formative assessment: Model ELISA in groups (20 min)
  - See appendix for sample instructions and sample models.
- Read introduction to lab and discuss as a class. (10 min)

- HW: Read lab instructions and answer prelab questions.
    - See appendix for sample scenario and prelab questions.

Day 4:
- Run ELISA investigation in lab groups.
- HW: lab write up

Day 5:
- Discuss ELISA results as a class. (30 min)
- Groups share results of their assigned patients.
- Connect back to immune system and news articles with possible discussion questions:
    - How can an ELISA test be used in response to a potential disease outbreak?
    - What real world challenges could limit the use of an ELISA test in a real disease outbreak scenario?
- Review immune system vocabulary (20 min)
- HW: Prepare for summative assessment.

Day 6:
Summative assessment

IX. Summative Assessment

Example rubric for lab write-ups

Requirements for Each Section
Pre Lab Questions (pts vary) Answered in complete sentences Completed prior to performing the lab experiment
Title (2 pts) Descriptive (states tested variables) Specific (states organism studied)
Purpose/Question (4 pts): Describes the purpose of experiment or question the experiment is designed to answer. Describes which variables are being tested. Explicitly states which is the independent vs. dependent variable. Written in the third person.
Hypothesis (3 pt) 1-2 sentences Predicts outcome of experiment Third person
Procedure (5 pts) Enough detail to allow for replication Past tense used

Data Tables (10 pts): Data tables are organized. Data tables are titled with descriptive titles. Correct units are present in the *headings* of the table.
Graphs (10 pts): All numerical data is presented in one or more graphs. Each graph is titled and both axes are labeled. Graph types (line, bar, etc) are appropriate for the data. Graph scales are appropriate for the data.
Conclusion (5 pts): 1-2 sentences stating results Specific, references data
Analysis (15pts): 4 short paragraphs addressing Possible reasoning for results are given. Explain the science behind your results. Potential sources of error are discussed. Further studies or experiments needed to confirm or provide further evidence are discussed. New additional questions

AP Assessment Questions

The following questions are publicly released free response questions from previous AP Biology Exams. Using released questions provides an opportunity for students to practice and gain confidence in answering the uniquely styled, multifaceted questions they will see on the AP exam in May. Because the questions have been released, there is also a risk that students can search for them in advance. Teachers can use their discretion to decide whether or not to incorporate released questions for graded class assessments.

2015 #8

An individual has lost the ability to activate B cells and mount a humoral immune response.

A. Propose ONE direct consequence of the loss of B-cell activity on the individual's humoral immune response to the initial exposure to a bacterial pathogen.
B. Propose ONE direct consequence of the loss of B-cell activity on the speed of the individual's humoral immune response to a second exposure to the bacterial pathogen.
C. Describe ONE characteristic of the individual's immune response to the bacterial pathogen that is not affected by the loss of B cells.

2007 #2 form B

An individual has lost the ability to activate B cells and mount a humoral immune response.

A. Propose ONE direct consequence of the loss of B-cell activity on the individual's humoral immune response to the initial exposure to a bacterial pathogen.
B. Propose ONE direct consequence of the loss of B-cell activity on the speed of the individual's humoral immune response to a second exposure to the bacterial pathogen.
C. Describe ONE characteristic of the individual's immune response to the bacterial pathogen that is not affected by the loss of B cells.

2005 #4

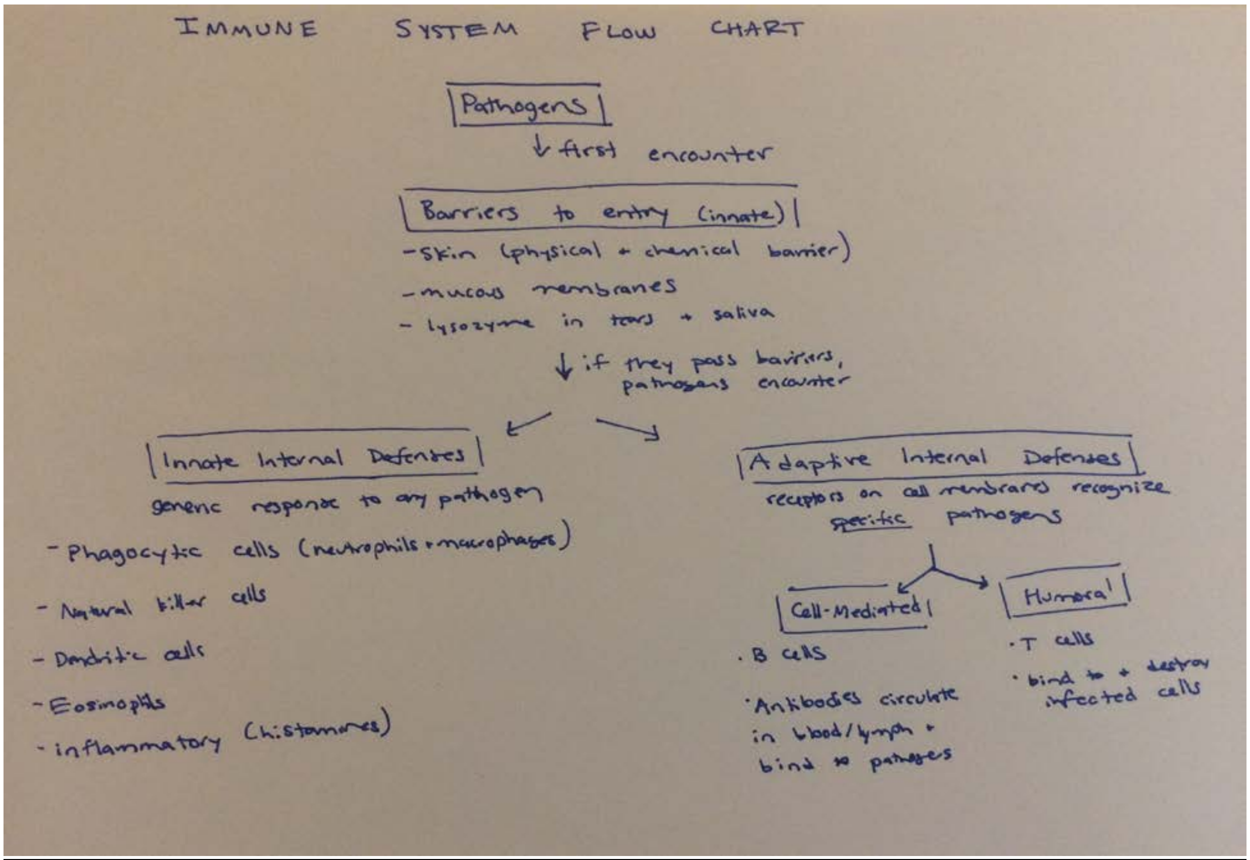
An individual has lost the ability to activate B cells and mount a humoral immune response.

A. Propose ONE direct consequence of the loss of B-cell activity on the individual's humoral immune response to the initial exposure to a bacterial pathogen.

B. Propose ONE direct consequence of the loss of B-cell activity on the speed of the individual's humoral immune response to a second exposure to the bacterial pathogen.
C. Describe ONE characteristic of the individual's immune response to the bacterial pathogen that is not affected by the loss of B cells.

Appendix- Sample Student Handouts

Formative Assessment Immune System Graphic Organizer



Reading Jigsaw Articles

Each group of students will read one article and share with their classmates. As the groups share, the listening students will record the major claim of each article, supporting evidence, and reasoning that explains the link between the evidence and the claim.

- Article regarding 2003 SARs outbreak.
  - https://harvardmagazine.com/2007/03/the-sars-scare.html
- Article regarding preemptive searches for new zoonotic viruses:
  - https://www.newsweek.com/new-sars-virus-discovered-myanmar-bats-1020810
- Article regarding simulation of government response to global pandemic:
  - https://www.businessinsider.com/pandemic-virus-simulation-johns-hopkins-shows-vulnerability-2018-7

Student Note Sheet

Article	Claim	Evidence	Reasoning
Harvard Magazine: SARs Outbreak			
Newsweek: PREDICT program			
Business Insider: CladeX Simulation			

ELISA Disease Exposure Investigation Handout

Background Information

A challenge of many laboratory techniques in immunology is that we are testing for substances that cannot be seen with the naked eye or even a microscope. Therefore, proxy measurements based on the interactions of molecules and chemical reactions must be used to determine the presence of a particular molecule. The ELISA technique allows us to determine the presence of an antigen based on whether or not binding to antibodies occurs.

Read the following webpage for more information before coming to class: Before class: **https://tinyurl.com/oz7l3ma**

Pre-Lab Modeling Activity

With your notes from class as a reference, use the available materials to work with your group members to create a model of an ELISA well that is positive for the presence of an antigen and an ELISA well that is negative for the presence of an antigen. Make sure that your model includes:

- Serum sample proteins

- Serum sample antigen
- Primary antibody
- Secondary antibody (with conjugated enzyme)
- Substrate

Keep in mind that each reagent is added to each well. Your model should show this but also show how the end result will differ between a positive and negative serum sample.

a. Share your model with your partner table and exchange feedback on how to improve the models.
b. Share your model with your teacher.
c. Sketch the positive and negative model in the space below and include a key stating what you used to represent each component of your model:

Disease Exposure Investigation – Using an ELISA to test for the presence of an antigen
Scenario was developed to be used with Protocol II of Biorad Immuno Explorer Kit: https://www.bio-rad.com/en-us/product/elisa-immuno-explorer-kit?ID=1e3f3100-99f6-49b3-b9a0-2c8aad9d9285

A group of twelve Westover students just returned from a two-week travel program. The program included sightseeing trips, as well as a five-day homestay which included volunteering at a local school. During the journey home, five students began complaining of headaches, sore throats, congestion, and coughs. It was initially assumed that the students had merely caught colds that were being exacerbated by the exhaustion of international travel and jet lag. However, CNN is reporting several cases of SARS have just been confirmed in the same town as the school where the Westover students volunteered. Could the students have contracted the virus and brought it to the US, or spread the virus to other passengers on their flights home?

Your challenge is to determine whether or not students were exposed to the SARs virus by using an ELISA assay to analyze their blood serum for the presence of the SARs antigen. After determining their exposure, you will devise a plan of action.
Before you read the lab manual protocol, consider the following questions:

1. Is it a waste of resources to test all twelve students if only five have symptoms? Why or why not?

2. Based on the above scenario, brainstorm ways in which students could have been exposed to the virus.

3. If students were exposed to the virus, who else is now at risk of contracting the disease? Explain.

<u>Sample ELISA models</u>