Are You What You Eat?
Detection of Body Fluid Contamination of Food

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Teacher Section

Overview

An antigen is any substance that triggers an immune response and production of antibodies. Antibodies (also called immunoglobulins) are produced by the immune system in response to an antigen. Antibodies reversibly bind to antigens through interactions such as hydrogen bonds and hydrophobic interactions. This biological process can be used to detect the presence of antigens using ELISA (enzyme linked immunosorbent assay).

Students will investigate the use of ELISA as a qualitative assay for detection of body fluid contamination of foods using protein L. Through the laboratory activities, students will see how antibodies interact with antigens with high affinity and specificity. The students will be able to apply this concept to the study of a new disease such as the PaV1 lobster virus.

This curriculum unit is appropriate to integrate into an immune system unit in High School Biology. Students will learn the chemistry and biology of ELISA. They will also learn about antigen/antibody binding within the body as well as its importance and implications in allergies and disease. Students will use the information they have gained to develop a lab protocol for studying a disease or allergy.

Science Background

Antigen-Antibody Binding

When an antigen enters an organism, the macrophages initiate the immune response by engulfing the antigen and displaying parts of the antigen to the Helper T-cells. Helper T-cells stimulate B-cells to divide rapidly. Helper T-cells also stimulate Killer T-cells and Memory T-cells. Some of the activated B-cells will become plasma cells, which produce antibodies while other B-cells will become memory cells, which divide rapidly upon reintroduction of the antigen.

Most antigen-antibody binding has high affinity, which is the strength of the attraction between the two molecules. Antigen-antibody binding also has high specificity. Specificity is the ability of an antibody to react with only specific antigen(s). Antibodies can distinguish differences in the primary, secondary, and tertiary structure of an antigen as well as isomers of an antigen. (3)

ELISA

ELISA is used to detect the presence of an antibody or antigen in a sample. To detect the presence of an antigen, the antibody specific for that antigen is used. To visualize, the antibody is conjugated with an enzyme that will cause a chromogenic substrate to change color. Horseradish peroxidase (HRP) is commonly conjugated to antibodies because they catalyze the oxidation of the substrate 3, 3', 5, 5'- tetramethylbenzidene (TMB). When oxidized, TMB is blue in color. The reaction is illustrated below:

3, 3', 5 5' Tetramethyl -benzidine + H_2O_2 —Horseradish-Peroxidase→ + $2\ H_2O$

Figure 1: Oxidation of TMB by HRP

(http://www.biosynth.com/media/reaktionsschemata/horseradish_peroxidase_100.jpg)

The first step of antibody capture ELISA is to coat the plate with the sample that may contain the antigen. The microplate wells are made of polystyrene which hydrophobically binds proteins. (2) Once coated, the plate is blocked using milk diluent so that no additional proteins will bind to the plate, such

as the conjugated antibody. The protein in the milk binds to any unused protein binding sites in the well, preventing nonspecific binding of the conjugated antibody. Next, the conjugated antibody is added. If any antigen is present in the well, it will bind. After washing the plate to remove conjugate that has not bound to the antigen, the substrate is added. If any conjugate remains in the well, it will produce a color change in the substrate.

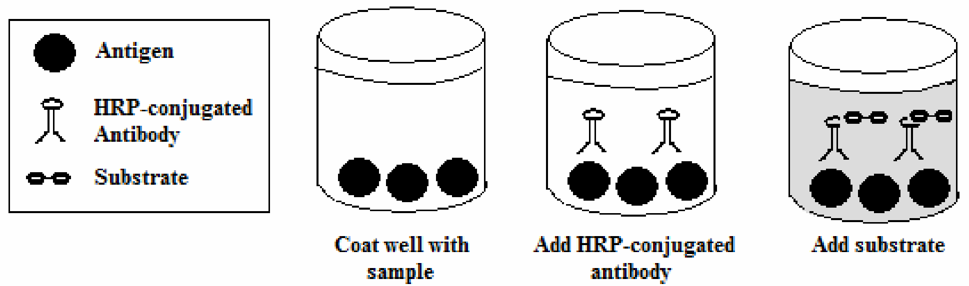


Figure 2: Major Steps of Antibody Capture ELISA

The plates can be read visually for qualitative data or using a microplate reader for quantitative data. Microplate readers detect the absorbance of light by the substrate. A standard curve must be run when using the microplate reader by performing a series of dilutions from a known concentration. This allows comparison of the sample wells to the standard curve which determines the concentration.

Controls are used to ensure that the procedure is working properly and can sometimes detect human error or contamination. In the negative control, the antigen is omitted. In the positive control, both the target antigen and the antibody are included.

ELISA and the Immune System

In this experiment, the antigen is HSP-protein L and the antibody is immunoglobulin. Protein L is expressed on the surface of *Peptostreptococcus magnus* and binds kappa light chain immunoglobulins, present in IgG, IgM, IgA, IgE, and IgD. (4) Protein L binds to Ig of species including humans, mice, rabbits, and rats. (1) The antibody binds with high affinity and specificity to protein L. This binding is visualized using TMB substrate.

Learning Objectives

- Students will learn how antigens and antibodies interact and students will demonstrate the concepts of specificity and affinity by playing a game that uses immunology terms and concepts.
- Students will investigate ELISA
- Students will learn how to use pipettes and micro-well plates.
- Students will learn how to perform a serial dilution.
- Students will qualitatively analyze their ELISA assays and interpret their data.
- Students will apply their knowledge of ELISA to the study of new diseases such as the PAV-1 lobster virus.

Time Requirements

For a 90 minute block period, about 6 days are needed to complete these lessons.

- Day 1: Antigen, Antibodies, and the Immune Response (Rh example)
- Day 2: ELISA background, Antibody production
- Day 3: Laboratory Procedures: Prepare solutions, practice pipetting and serial dilutions
- Day 4: Body Fluid Detection using Protein L
- Day 5: Student Protocol Development, Real-World Applications

Advance Preparation

Antigen-Antibody Game

- Materials:
  - Immunologic Cells: antibody (Immunoglobulin), macrophage, Helper T-cell, B-cell, Plasma Cell, Memory B-cell, Memory T-cell, Killer T-cell
  - Antigens: various shapes complementary to the antibody shapes, viruses
- Directions:
  - Before class, hide antigens and viruses around the room or activity area
  - Pass out the assigned immunologic cells
  - B-cell students will move around the room to find the shape complementary to theirs (lock and key fit) while macrophages search for anything foreign (especially the viruses)
  - Once found, the B-cell and macrophage students "call for back-up" from the other immunologic cells
- Prep Time: allow 15 minutes to make the cells and antigens and allow 5 minutes to place antigens around the classroom or activity area.

Demo ELISA plate

- Materials:
  - Microplate
  - Food coloring
- Directions:
  - Prepare a sample ELISA plate using blue food coloring to represent a positive result
  - Be sure to include the following:
    - a serial dilution for the standard curve
    - positive control
    - negative control
    - several positive and negative results, run in duplicates
- Prep Time: 5 minutes

ELISA Lab: Protein L

- Directions for preparing reagents:
  - 100 mL Carbonate Buffer (pH 9.6-9.7)
    - 15mM Na_2CO_3 (0.16 g)
    - 35mM $NaHCO_3$ (0.29 g)
    - H_2O to 0.1 L
  - 2 L PBS/Tween-20
    - 1.9 mM NaH_2PO_4 (0.456 g)
    - 8.1 mM $Na2HPO_4$ (2.300 g)
    - 154 mM NaCl (17.900 g)
    - Add H_2O to ~200mL less than 2L
    - Adjust pH to 7.2-7.4 using 1M NaOH or 1M HCL
    - Add H_2O to 2L
    - Add Tween-20 to 0.05% of final volume (1mL)

    - Blocking Solution
        - 875µL Milk Block Stock (20X)
        - 16.6 mL PBS/Tween-20

- Unknown Sample Collection:
    - Materials and Equipment:
        - 6 microfuge tubes
        - 3 mL carbonate buffer
        - Spoons
    - Label each spoon and record what each was spiked with
        - Dispense 0.5mL of (varying concentrations of mouse serum or human saliva)
            - If using human serum, be sure to follow biohazard procedures
        - Allow samples to dry
- Materials:
    - 5µL mouse serum
    - 5mL Carbonate Buffer (pH 9.6-9.7)
    - 240mL PBS/Tween-20 (pH 7.2-7.4)
    - Antigen Samples (collected previously)
    - 10µL Conjugate (primary antibody): HRP-Protein L
    - 437µL Blocking solution
    - 8.2 mL TMB Membrane Peroxidase Substrate System (c, c', 5, 5'-tetramethylbenzidine)
- Equipment:
    - 2 96-well plates
    - Reagent boats
    - Microfuge tubes 0.5-2mL with rack
    - Pipette tips
    - Pipettes: multichannel, 1 mL, and 5-50µL
    - Plastic wrap
    - Microfuge tubes with rack
- Prep time: *15 minutes to prepare spoons for sample collection*

Materials and Equipment for a class of 20 students (10 groups of 2 students)

Complete List of Materials and Equipment:

- 2L Carbonate Buffer (pH 9.6-9.7)
    - 3.2g Na_2CO_3
    - 5.8g $NaHCO_3$
- 200L PBS/Tween-20 (pH 7.2-7.4)
    - 9.12g NaH_2PO_4
    - 46g Na_2HPO_4
    - 358g NaCl
    - 20mL Tween-20
    - 20mL 1M NaOH
    - 20mL 1M HCl
- Antigen: 10µL mouse serum
- Conjugate (primary antibody): 20µL HRP-Protein L
- 60L DI water pH 7.0-7.4

- 15 mL Blocking solution
- 16.5 mL TMB Membrane Peroxidase Substrate System (c, c', 5, 5'-tetramethylbenzidine)
- pH meter
- 2L Beaker
- 100mL Beaker
- 1 L Graduated Cylinder
- 2 96-well plate (additional ones can be used for pipetting and serial dilution practice)
- Reagent boats
- Microfuge tubes 0.5-2mL with rack
- Pipette tips
- Pipettes: 1mL, 5-50μL, and multichannel
- Plastic wrap
- Blue food coloring
- Weighing paper
- Scoopula
- Triple Beam or Electronic balance

Cost Estimate and Purchasing: (estimate assumes the glassware, reagent boats, pipettes, pipette tips, pH meter, and microfuge tubes do not need to be purchased)

Item	Cost	Supplier	Catalog No.
Sodium Carbonate, Na_2CO_3 (**CAS No.** 497-19-8)	$9.60/500g	Ward's	942 V 9006
Sodium Bicarbonate, $NaHCO_3$ (**CAS No.** 144-55-8)	$6.30/500g	Ward's	942 V 7606
Sodium Phosphate-monobasic-anhydrous, NaH_2PO_4 (**CAS No.** 7558-80-7)	$15.50/500g	Ward's	943 V 3306
Sodium Phosphate-dibasic-anhydrous, Na_2HPO_4 (**CAS No.** 7558-79-4)	$11.50/100g	Ward's	943 V 2904
Sodium Chloride, NaCl (**CAS No.** 7647-14-5)	$6.20/500g	Ward's	942 V 9706
Tween-20	$6.95/30mL	B&H	PHT20S30ML
Sodium Hydroxide, 1M NaOH (**CAS No.** 1310-73-2)	$6.50/500mL	Ward's	970 V 8006
Hydrochloric Acid, 1M HCl (**CAS No.** 7647-01-0)	$5.70/500mL	Ward's	970 V 3606
Mouse IgG	$96.30/50mg	Sigma	15381
HRP-Protein L	$136.00/0.5mg	Pierce	32420
Blocking solution	$92.80/200mL	KPL	50-82-01
TMB Membrane Peroxidase Substrate System	$45.00/100 mL	KPL	50-77-18
Microplates ELISA, round, PVC	$34.55/50 per case	Fisher	08-772-8
TOTAL	$472.90		

Precautions and Chemical storage:

Handle the 1M HCl and 1M NaOH with care. Store the HCl in a designated acids cabinet and store the NaOH in a dedicated corrosives cabinet. The HRP-Protein L, Mouse Serum, Blocking solution, and TMB all should be stored in a dedicated chemical refrigerator.

Student Prior Knowledge and Skills

Students should be able to:

- pipette
- use linear algebraic equations to solve concentration/volume calculations
- convert between metric unit prefixes, including nano-
- understand basic laboratory safety procedures
- locate safety equipment such as an eyewash station, first aid kit, telephone, spill kit, and fire extinguisher
- wear appropriate laboratory attire and come dressed accordingly
- take volume and mass measurements, checking twice for accuracy
- tare the balance before taking mass measurements
- keep a clean laboratory bench
- clean all glassware before and after a laboratory exercise

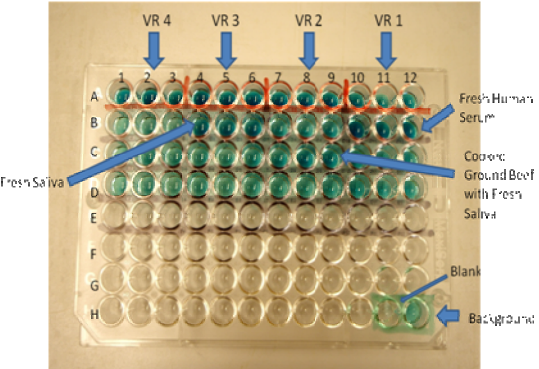
What is Expected from Students

Students should:

- actively participate in the Antigen-Antibody Game
- complete the Protein L laboratory exercise
- analyze the data and submit the results in the form of a laboratory report
- design a laboratory protocol to test for a specific antigen or antibody as a means of studying a new disease

Anticipated Results

- Example results for the Mammalian IgG Lab (individual results will vary based on sample selection)



- Source of error: letting substrate sit too long
    - The TMB substrate is light sensitive. Overexposure to light or prolonged incubation causes nonspecific color development or the TMB precipitates out of solution.

Lesson Plans

Day 1: Antigens, Antibodies, and the Immune Response (Rh example)

- Engagement:
    - If your house was going to be attacked tomorrow, what preparations would you take today to defend it?
- Exploration:
    - Dig Deeper:
        - What will you do if the enemy breaks through your barriers?

        - How will you communicate to your teammates?
        - How will you know who is on your team?
        - Once the enemy has succeeded in a certain attack, how will you prevent that from occurring again?
    - Explain how antigens, macrophages, B cells, T cells, and antibodies (immunoglobulins) fit into an attack scenario. Assign a military role to each immune system cell.
- Explanation:
    - Antigens: any foreign substance that triggers an immune response
    - Antibodies (immunoglobulins): glycoprotein secreted by plasma cells, bind with high affinity and specificity to antigens
        - Classes: IgA, IgD, IgE, IgG, IgM
    - Stages of the Immune Response:
        - Recognition of virus (macrophage) or bacteria (B cells)
        - Activation: presentation to Helper T cell
        - Proliferation of Killer T cells if viral, proliferation of B cells to make antibodies if bacterial
            - Antibodies deactivate and/or promote phagocytosis by macrophage
        - Defeat of Antigen
        - Down-regulation of response
        - Memory T or B cells
            - If the same antigen presents itself, the body will be prepared to attack quicker
            - Usually the first response causes mild symptoms of an elevated immune system because the attack is slow but steady; in a second exposure, the attach is much faster and stronger
    - Antigen-Antibody Game
- Elaboration:
    - Rh is a protein that is sometimes present on the surface of red blood cells
        - If you carry this protein, you are Rh-positive; if you do not, you are Rh-negative
    - It is possible for an Rh-negative mother to have an Rh-positive fetus
        - Not a problem for the first child but if there is a placental tear during this childbirth or other mixing of the blood of the mother and fetus, the Rh-negative mother is exposed to the Rh protein
    - Explain what happens to the mother after the exposure to the Rh protein
    - What might happen if the mother conceives a second Rh-positive child?
- Evaluation:
    - Self-Assessment: 3 key ideas I learned today, 2 vocabulary definitions, 1 question I still have
    - Discussion Question: Will there be an immune response if an Rh-positive person is exposed to Rh-negative blood (assuming same ABO type)?

Day 2: ELISA background, How antibodies are created in a laboratory

- Engagement:
    - Define the following: aliquot, assay, HRP, serial dilution, and substrate
    - Identify the following equipment: pipette, pipette tip, microplate
- Exploration:
    - Give students example ELISA plates
        - Tell students that the antigen is on the surface of the plastic plate…have them sketch this

        - Have students predict and sketch the placement of the antibody
        - Have students brainstorm why there is a color change and what it might mean
        - Have students brainstorm the purpose of the standard curve
- Explanation:
    - ELISA (enzyme linked immunosorbent assay) is used to detect the presence of an antibody or antigen in a sample
    - In ELISA, the antibody is conjugated with an enzyme that will cause a chromogenic substrate to change color (Horseradish peroxidase, HRP)
        - HRP catalyzes the oxidation of the substrate TMB (turns blue)
    - Steps:
        - Coat the plate with the sample that may contain the antigen
        - Block using milk diluent so that no additional proteins will bind to the plate
            - prevents nonspecific binding of the conjugated antibody
        - Add conjugated antibody, binds to antigen if present
        - Wash to remove unbound conjugate
        - Add substrate, if any conjugate remains in the well, it will produce a color change in the substrate.
        - Read plate visually
            - Discuss purpose of standard curve and +/- controls
    - Antibodies to a known antigen can be created in the laboratory
        - inject animal with a known antigen (such as IgG), causes an initial immune response
        - collect antibodies, conjugate with HRP to allow detection using TMB substrate
- Elaboration:
    - Explain how you could prepare a presumptive test for human blood
    - Do you think it would be specific for humans or might you get some response from other mammals?
    - Take swabs of food and food surfaces for use in a later lab (store in refrigerator)
- Evaluation:
    - Self-Assessment: What did I learn today? What am I confused about?
    - Discussion Question: Why is the milk block step important? What might your results look like if you forget this step?
    - Assessment:
        - What substance produces the color change?
        - How would I know by looking at the results that I forgot to add substrate? Would skipping the conjugate cause the same result?
        - What do I compare the results to in order to determine a positive or negative result?
        - How would I prepare an antibody against mouse IgA?

Day 3: Prepare solutions, practice pipetting and serial dilutions

- Engagement:
    - Use stoichiometry to convert the following mM values to grams (hint: you will need to calculate the molar volume (g/L) of the compound)
        - 15 mM Na_2CO_3
        - 35 mM $NaHCO_3$
        - 1.9mM NaH_2PO_4
        - 8.1 mM Na_2HPO_4

        - 154 mM NaCl
    - Follow the procedure to prepare the carbonate buffer and PBS
- Exploration:
    - Serial Dilution Example using blue food coloring:
        - Fill the first well with 55 μl of initial solution
        - Fill the rest of the row with 45μL of water
        - Transfer 5μL from the first to the second and mix. Transfer 5μL to the next well, continue.
    - What is the Dilution? *(Answer 5:50 or 1:10)*
    - How many wells would you need to dilute the original sample to 1:1,000,000?
- Explanation/Elaboration:
    - Assume you have a sample that has a concentration of 1mg/mL
        - What is the new concentration? ($C_1V_1=C_2V_2$) of each well in the serial dilution?
    - Draw Plate layout, be sure to include standard curve, positive control, and negative control
        - What should the positive control have in it?
        - What should the negative control have in it?
        - What is the dilution factor in well A5?
- Evaluation:
    - Self-Assessment:
        - How was my pipetting technique?
        - What concept did I not understand?
    - Discussion Question: If while pipetting the standard curve, you had an air bubble in the pipette tip, would it affect the curve? If so, how? If not, why not?

Day 4: Complete Protein L Plate

- Engagement/Exploration:
    - Protein L is specific for Ig in humans, mice, rats, and rabbits.
        - Is protein L the antigen or antibody?
        - Is Ig the antigen or antibody?
        - Predict the plate layout
- Elaboration:
    - Performing the coating step for the Mammalian Ig plate
- Evaluation:
    - Prepare Formal Laboratory report on results of Mammalian IgG plate (use rubric)

Day 5: Student Protocol Development, Reading/Writing in Science, Real-World Applications

- Engagement/Exploration:
    - What would you like to be able to detect? Why?
        - Examples: HIV, SARS, Lyme Disease, FLV, other viruses, mold toxins, presence of peanuts in foods, illicit drugs, anthrax, E.Coli, hCG (pregnancy)
    - How would you make your conjugated antibody for this assay?
    - Draw what your well would look like, identifying your antigen and antibody
- Elaboration:
    - Develop a protocol for your ELISA assay. Be sure to include a graphic that illustrates the well arrangement.
- Evaluation:
    - Discussion Questions:
        - How could you use ELISA to detect an allergy? What molecule will the HSP tag need be on?

- How might ELISA be used to screen for biological warfare agents?
  - More info at http://www.bt.cdc.gov/bioterrorism/overview.asp
- How might ELISA be used to detect a new/unrecognized virus?
- Presumptive drug testing can be performed using ELISA. The plates are coated with a primary antibody. Drug will bind to the antibody if present. A conjugated secondary antibody is added. The drug and the secondary antibody compete for binding sites on the primary antibody. If the sample is positive, would you expect a clear or a blue well? Illustrate the arrangement of the molecules in the well. Be sure to label each molecule.

Assessment

- Assessment:
  - Self-Assessment
  - Discussion Questions
  - Laboratory Results
  - Post-lab Questions
  - Formal Laboratory Report
  - Self-Created ELISA protocol
- Formal Laboratory Report (with rubric)
- Student-Created ELISA protocol Rubric

Student Section

Rationale

We have all had to get vaccines. A vaccine introduces a small or innocuous amount of a virus into our bodies so that we will initiate an immune response to it. The vaccine helps most people avoid the disease because the immune system remembers that “bug” and can attack immediately if it meets it again. So what does that have to do with detecting mammalian blood or saliva on food?

Introduction

The immune system contains five classes of immunoglobulins (antibodies) that bind with specific antigens. Once a new antigen is exposed to an organism, the immune system will launch an attack and maintain a memory of the attack. This process allows scientists to manufacture antibodies against very specific antigens.

Over the next few days, you will learn how this biological process is used in ELISA (enzyme linked immunosorbent assay) to detect antigens or antibodies. You will see this process in the laboratory exercise using the antigen Mouse IgG and HRP-Protein L. The HRP stands for horseradish peroxidase, which causes a colorimetric reaction that allows for visualization of the antigen-antibody binding. You will use your knowledge of ELISA to create a protocol to detect an antigen of your choice.

Background

Antigen-Antibody Binding

When an antigen enters an organism, the macrophages initiate the immune response by engulfing the antigen and displaying parts of the antigen to the Helper T-cells. Helper T-cells stimulate B-cells to divide rapidly. Helper T-cells also stimulate Killer T-cells and Memory T-cells. Some of the activated B-cells will become plasma cells, which produce antibodies while other B-cells will become memory cells, which divide rapidly upon reintroduction of the antigen.

Most antigen-antibody binding has high affinity, which is the strength of the attraction between the two molecules. Antigen-antibody binding also has high specificity. Specificity is the ability of an antibody to react with only specific antigen(s). Antibodies can distinguish differences in the primary, secondary, and tertiary structure of an antigen as well as isomers of an antigen. (3)

ELISA

ELISA is used to detect the presence of an antibody or antigen in a sample. To detect the presence of an antigen, the antibody specific for that antigen is used. To visualize, the antibody is conjugated with an enzyme that will cause a chromogenic substrate to change color. Horseradish peroxidase (HRP) is commonly conjugated to antibodies because they catalyze the oxidation of the substrate 3, 3’, 5, 5’- tetramethylbenzidene (TMB). When oxidized, TMB is blue in color. The reaction is illustrated below:

Figure 1: Oxidation of TMB by HRP

(http://www.biosynth.com/media/reaktionsschemata/horseradish_peroxidase_100.jpg)

The first step of antibody capture ELISA is to coat the plate with the sample that may contain the antigen. The microplate wells are made of polystyrene which hydrophobically binds proteins. (2) Once coated, the plate is blocked using milk diluent so that no additional proteins will bind to the plate, such as the conjugated antibody. The protein in the milk binds to any unused protein binding sites in the well, preventing nonspecific binding of the conjugated antibody. Next, the conjugated antibody is added. If

any antigen is present in the well, it will bind. After washing the plate to remove conjugate that has not bound to the antigen, the substrate is added. If any conjugate remains in the well, it will produce a color change in the substrate.

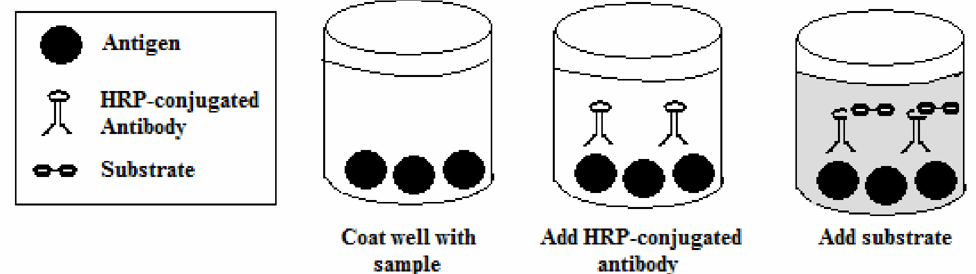


Figure 2: Major Steps of Antibody Capture ELISA

The plates can be read visually for qualitative data or using a microplate reader for quantitative data. Microplate readers detect the absorbance of light by the substrate. A standard curve is made by performing a series of dilutions from a known concentration. This allows comparison of the sample wells to the standard curve which determines the relative concentration.

Controls are used to ensure that the procedure is working properly and can sometimes detect human error or contamination. In the negative control, the antigen is omitted. In the positive control, both the target antigen and the antibody are included.

ELISA and the Immune System

In this experiment, the antigen is mammalian antibodies and the antibody is HSP-protein L. Protein L is expressed on the surface of *Peptostreptococcus magnus* and binds kappa light chain immunoglobulins, present in IgG, IgM, IgA, IgE, and IgD. (4) Protein L is binds to Ig of species including humans, mice, rabbits, and rats. (1) The antibody binds with high affinity and specificity to protein L. This binding is visualized using TMB substrate.

Name:____ Date: ________________

Vocabulary:

Affinity: ____

Aliquot: ____

Antibody: ____

Antigen: ____

Assay: ____

B Cell: ____

Chromogenic: ____

Contamination: ____

Dilution: ____

Enzyme Linked Immunosorbent Assay: ____

Helper T Cell: ____

HRP: ____

Hydrophobic: ____

Immune System: ____

Immunoglobulin: ____

Killer T Cell: ____

Macrophage: ____

Memory B Cell: ____

Memory T Cell: ____

Negative Control: ____

Pipette: ____

Plasma Cell: ____

Positive Control: ____

Specificity: ____

Standard Curve: ____

Name:____ Date: _________________

Antigen-Antibody Binding Game

Stages of the Immune Response:

1. **Recognition** of virus (by the macrophage) or bacteria (by the B-cells)
2. **Activation**: presentation to Helper-T cell
3. **Proliferation** of Killer T cells if viral, **proliferation** of B cells to make antibodies if bacterial
    a. Antibodies deactivate and/or promote phagocytosis by macrophage
4. **Defeat** of Antigen
5. **Down-regulation** of response
6. **Memory** T or B cells
    a. If the same antigen presents itself, the body will be prepared to attack the antigen quicker
    b. Usually the first response causes mild symptoms because the attack on the invader is slow but steady; in a second exposure, the attack is much faster and stronger

Prelab Questions

1. Obtain an immunologic cell from the teacher (you will be one of the following: antibody, macrophage, Helper-T cell, B-Cell, Plasma cell, Memory B-cell, Memory T-cell, Killer-T cell)
    a. What is the function of your immunologic cell? ___________________________________

2. The B-cells will move around the room searching for their particular antigen. When they find it, they will bind to it very tightly. These two concepts are called ___________________ and _____________________. Once a B-cell has found a bacteria, it will summon the assistance of ___________________________.

3. The macrophages will move around the room searching for any foreign objects to engulf. If the macrophage encounters a virus, it will summon the help of ____________________________.

Procedure

1. If you are a B-cell or a macrophage, begin your search. If not, wait until you are summoned by the appropriate immunologic cell.
2. When summoned, arrive at the area and describe what your role is and, if necessary, pass the message along to the next immunologic cell in the immune response chain.

Post-lab Questions

1. What was your role in the game today?
____
____

2. The virus HIV inactivates Killer-T cells. How does this disrupt the immune response?
____
____

Name:____ Date: _______________

Collection of Samples for Detection of Immunoglobulins

Materials and Equipment:

- 6 Microfuge tubes per lab group
- 3 mL Carbonate Buffer per lab group
- Disposable Pipette

Safety Note: Be sure to wear gloves when collecting samples.

Procedure:

1. Number your microfuge tubes 1-6.
2. Dispense 0.5 mL carbonate into the spoon suspected to contain immunoglobulins. Gently move the spoon around to dissolve any immunoglobulins that may be present.
3. Use a disposable pipette to remove the liquid from the swab and dispense it into the microfuge tube.
4. Place the microfuge tubes in a rack and place the rack in the refrigerator. Do not store samples for more than two days before testing.

Data Table:

Tube Number	Collection Location/Description	Collection Date

Name:____ Date: ________________

Detection of Contamination using ELISA

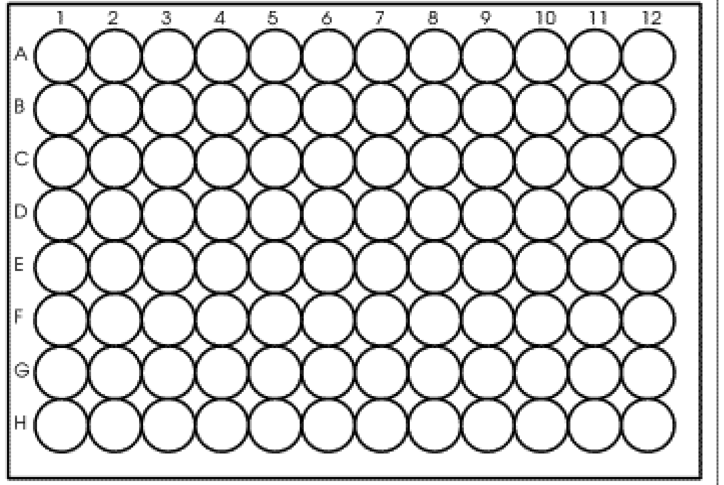
Reagent List
5μL Mouse Serum
5mL Carbonate buffer
240mL PBS/Tween-20
Contamination samples (previously collected)
10μL HRP- Protein L
8.2mL TMB substrate
450μL Milk Block Diluent

Equipment List
5-50μL and 1mL pipette
multichannel pipette
6 microfuge tubes
pipette tips
Plastic wrap
5 Reagent boats
96-well plate

Safety Precaution: Wear gloves when handling all reagents.

Prelab:

1. Draw plate layout below after reading the protocol:



2. Is protein L the antigen or antibody? _____________ Is Ig the antigen or antibody? __________
3. Complete the following table:

Visual Rating	Dilution Factor	Concentration
4	1:	ng/mL
3	1:	ng/mL
2	1:	ng/mL
1	1:	ng/mL

Procedure:

1. Prepare the Visual Standard in labeled microfuge tubes as follows:
   Visual Rating 4 — 5μL Mouse Serum : 995μL carbonate
   Visual Rating 3 — 5μL Visual Rating #4 : 145μL carbonate
   Visual Rating 2 — 5μL Visual Rating #4 : 320μL carbonate
   Visual Rating 1 — 100μL Visual Rating #2 : 100μL carbonate

2. Pipette 50µL of VR 4 into wells A3 and A4
   Pipette 50µL of VR 3 into wells A5 and A6
   Pipette 50µL of VR 2 into wells A7 and A8
   Pipette 50µL of VR 1 into wells A9 and A10
   Pipette 50µL of carbonate buffer into the blank and background (wells A1 and A2)
   Pipette 50µL of samples, recording well location and description below:

Sample #	Location	Description	Visual Rating	Approximate Concentration
				ng/mL
				ng/mL
				ng/mL
				ng/mL
				ng/mL
				ng/mL

3. Wrap the plate in plastic wrap and label your plate with your name and your partner's name. Incubate overnight at room temperature.

4. Rinse plate with 200µL PBS/Tween-20 four times using a 12 or 8 multi-channel pipette.

5. While one laboratory partner rinses the plate, the other should prepare the milk block diluent. The milk block diluent is prepared by adding 437µL milk block to 8.3mL PBS/Tween-20. Dispense 200µL milk block solution. Incubate room temperature for 30 minutes. Gently mix by swirling the plate while placed on a flat surface.

6. Rinse plate with 200µL PBS/Tween-20 four times using a 12 or 8 multi-channel pipette.

7. While one laboratory partner rinses the plate, the other should prepare the protein L dilution. You will pipette 50µL protein L at 1:500 to all wells **<u>except</u>** the blank. To prepare a 1:500 dilution, in a microfuge tube add 10µL protein L to __________µL carbonate buffer. (Remember to subtract the 10µL from your total to determine the µL of carbonate buffer needed!) Incubate at room temperature by mixing gently for 30 minutes.

8. Rinse plate with 200µL PBS/Tween-20 four times using a 12 or 8 multi-channel pipette.

9. While one laboratory partner rinses the plate, the other should prepare the TMB substrate. This can be prepared by adding 4.10 mL TMB to 4.10mL Solution B. Pipette 75µL substrate to each well and incubate room temperature for 10 minutes.

10. Read the plate by comparing your samples to the visual standard. Assign a visual rating based on the color intensity. Be sure to note the color of the blank and background as well. Determine the approximate concentration of your samples based on the visual rating.

Discussion/Analysis

1. If the protein L was not HRP-conjugated, would it still bind to the immunoglobulins? Explain.

2. What molecule is the HSP tag on? Is this the antigen or antibody?

3. How were you able to determine the approximate concentration of the samples?

4. In this laboratory, we used normal mouse serum. Based on the activity of protein L, could this experiment detect human blood contamination? _____________
   Bovine (cow) blood contamination? ________________________
   Goat blood contamination? _______________________________
   Chicken blood contamination? _____________________________
   Mouse blood contamination? ______________________________

5. If the negative control had shown a visual rating of 3, what could we conclude about our results?

Name:____ Date: ________________

Student-Created ELISA Protocol

If you could detect any virus, bacteria, or drug in the world, what would you choose? (Examples: HIV, SARS, Lyme's Disease)

Describe how you would make an antibody for this assay:

What would the microplate well look like with the antigen and antibody bound? Label the antigen and antibody.

Develop a protocol for your ELISA assay. Be sure to include a graphic that illustrates the plate arrangement. (see rubric)

Discussion Questions:

1. How could you use ELISA to detect an allergy? What molecule will the HSP tag need be on?

2. How might ELISA be used to screen for biological warfare agents? (More info at http://www.bt.cdc.gov/bioterrorism/overview.asp)

3. How might ELISA be used to detect a new/unrecognized virus? How would scientists create an antibody for this virus? *Read the article: PaV1.*

4. Presumptive drug testing can be performed using ELISA. The plates are coated with a primary antibody. Drug will bind to the antibody if present. A conjugated secondary antibody is added. The drug and the secondary antibody compete for binding sites on the primary antibody. If the sample is positive, would you expect a clear or a blue well? Illustrate the arrangement of the molecules in the well. Be sure to label each molecule.

Detection of Contamination using ELISA: Formal Lab Report Rubric

Task	Bulls-eye	Near Miss	On the Board	Keep Trying!
Title and Background	-Title clearly indicates objective - Detailed background with strong introduction -Appropriate diagrams are included -Your name and your lab partner(s) name is present	-Appropriate title -Sufficient background and introduction -Some diagrams -You and your lab partner(s) name is present	-Title not informative -Insufficient background, poor introduction -Few /inappropriate diagrams included	-No title -Background grossly insufficient -Very inappropriate diagrams included if any at all
Hypothesis	-Hypothesis identifies independent and dependent variables and the control	-Hypothesis identifies the independent and dependent variables	-Hypothesis is incomplete/poorly written	-Hypothesis is missing or unclear
Materials	-Materials list is complete and identifies amounts of each item -includes necessary equipment	-Complete materials list with necessary equipment	-Materials/ equipment list is incomplete	-Materials list is very incomplete
Protocol	-Information and directions are very clear -Safety Precautions are identified -Valid test of variables -Has instructions for data collection	-Clear information and directions -Safety precautions are identified -Valid test of variables -Has instructions for data collection	-Information and directions are unclear and/or missing components -Safety precautions are insufficient/ missing	-Little information and directions -Not possible to replicate experiment using this protocol
Results	-Data is excellently presented in organized and labeled charts using the appropriate units -Data is summarized in paragraph format -All calculations are shown	-Data is organized in charts using the appropriate units -Data is summarized in paragraph format -Some calculations	-Data is incomplete or incorrectly represented in charts -Poor data summary	-Data not represented in charts or grossly incorrect -Poor/missing data summary
Discussion and Conclusion	-Results are completely discussed -Any discrepancies are explained -Relationship between results and hypothesis clearly explained -Purpose of experiment stated -Sources of error are identified -Conclusion shows that you understood the scientific concepts involved -Discussion of future research that may aide understanding of results	Contains 6 out of the seven components	Contains 4 or 5 of the seven components	Contains 3 or less of the seven components
Format	- Report was neat and legible - Report was neatly typed, doubled spaced, one inch margins and 12 point font -Report used correct spelling, grammar and sentence structure -Personal pronouns are not used -Sections are titled -Report was submitted on time -Report was very challenging to your abilities	Most criteria met -Report was challenging to your abilities	Some criteria met -Report was appropriate to your abilities	Few criteria met -Report was not up to your ability

Student-Created ELISA Protocol Rubric

Task	Bulls-eye	Near Miss	On the Board	Keep Trying!
Title and Background	-Title clearly indicates objective - Detailed background with strong introduction -Appropriate diagrams are included	-Appropriate title -Sufficient background and introduction -Some diagrams	-Title not informative -Insufficient background, poor introduction -Few /inappropriate diagrams included	-No title -Background grossly insufficient -Very inappropriate diagrams included if any at all
Hypothesis	-Hypothesis identifies independent and dependent variables and the control	-Hypothesis identifies the independent and dependent variables	-Hypothesis is incomplete/poorly written	-Hypothesis is missing or unclear
Materials	-Materials list is complete -includes necessary equipment	-Mostly complete materials list with necessary equipment	-Materials/ equipment list is incomplete	-Materials list is very incomplete
Protocol	-Information and directions are very clear -Safety Precautions are identified -Valid test of variables -Has instructions for data collection	-Clear information and directions -Safety precautions are identified -Valid test of variables -Has instructions for data collection	-Information and directions are unclear and/or missing components -Safety precautions are insufficient/ missing	-Little information and directions -Not possible to replicate experiment using this protocol
Format	- Report was neat and legible - Report was neatly typed, doubled spaced, one inch margins and 12 point font -Report used correct spelling, grammar and sentence structure -Personal pronouns are not used -Sections are titled -Report was submitted on time -Report was very challenging to your abilities	Most criteria met -Report was challenging to your abilities	Some criteria met -Report was appropriate to your abilities	Few criteria met -Report was not up to your ability

References

1. http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-3083.1993.tb03310.x
2. "Biotechnology Explorer, Elisa Immuno Explorer Kit Instruction Manual" BioRad.
3. http://pathmicro.med.sc.edu/mayer/ab-ag-rx.htm
4. http://www.genscript.com/product_001/molecule/code/M00098/category/molecule/HRP_Protein_L.html

Immune System Links

http://bcs.whfreeman.com/thelifewire/content/chp18/1802001.html