Immunodeficiencies:
From Animal Models to the High School Classroom

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Teacher Guide

I. Science Background

The immune system is a complex system of cells and tissues that must work in harmony to protect us from infections. It is vital to our health that our immune system functions properly. Defects lead to reduced host defense and serious infections, which are termed as immunodeficiency diseases. Immunodeficiency can be a result of genetic mutations in immune cells or tissues, or can be induced by certain medications or infections, such as HIV infection. This curriculum includes an introduction to the immune system, genetic engineering of knockout mice, and how animal models are used to investigate the roles of the immune system in particular diseases. Students will then dive into laboratory techniques, including enzyme-linked immunosorbent assay (ELISA) to detect serum antibody levels in mouse blood and flow cytometry to phenotype wild type mice versus CD4 T cell knockout mice. The flow cytometry will occur in my mentor's laboratory at the University of New England, while the ELISA will be performed in the classroom. The unit will conclude with facilitating students to use what they have learned about the immune system to examine the consequences following disruptions of specific immune components, thus further understanding particular immunodeficiency diseases. Student groups will create a poster and present it to the class and other biology classes at the school. The goal of this unit is to 1) enhance students' understanding of how animals are used as disease models in research and 2) to learn how defects in the immune response can be detected using laboratory techniques.

II. Student Outcomes

A. Science Concepts Covered

- Immunity: Innate versus Adaptive Immunity
- Immune Cell Types and Signaling Molecules
- Cell Interactions and Systems
- Immunodeficiency Disorders
- Genetic Engineering

B. Course Placement

This unit was planned for an AP Biology class to cover the immune system and cell interactions and signaling as part of the AP course but can be used in an honors or college prep level genetics or biotechnology class.

C. AP Biology Curriculum Standards and Practices

2.D.4: Plants and animals have a variety of chemical defenses against infections that

affect dynamic homeostasis.
2.E.1: Timing and coordination of specific events are necessary for the normal development of an organism, and these events are regulated by a variety of mechanisms.
3.A.1: DNA, and in some cases RNA, is the primary source of heritable information.
3.A.3: The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.
3.B.2: A variety of intercellular and intracellular signal transmissions mediate gene expression.
3.D.1: Cell communication processes share common features that reflect a shared evolutionary history.
3.D.2: Cells communicate with each other through direct contact with other cells or from a distance via chemical signaling.
3.D.3: Signal transduction pathways link signal reception with cellular responses.
3.D.4: Changes in signal transduction pathways can alter cellular response.
3.E: Transmission of information results in changes within and between biological systems.
4.B.1: Interactions between molecules affect their structure and function.

Science Practices

SP1: The student can use representations and models to communicate scientific phenomena and solve scientific problems. (**1.1, 1.2, 1.4, 1.5)**
SP2: The student can use mathematics appropriately. (**2.2)**
SP5: The student can perform data analysis and evaluation of evidence. **(5.3)**
SP6: The student can work with scientific explanations and theories. (**6.1, 6.2, 6.4)**
SP7: The student is able to connect and relate knowledge across various scales, concepts and representations in and across domains. (**7.1)**

D. Next Generation Science Standards (NGSS)

HS-LS-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

LS1.A: Structure and Function
Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS-1)

HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.

LS1.A: Structure and Function

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. (HS-LS1-2)

HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

LS3.A: Inheritance of Traits

Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no known function. (HS-LS3-1)

HS-LS3-2. Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

LS3.B: Variation of Traits

In sexual reproduction, chromosomes can sometimes swap sections during the process of meiosis (cell division), thereby creating new genetic combinations and thus more genetic variation. Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. Environmental factors can also cause mutations in genes, and viable mutations are inherited. (HS-LS3-2)

III. Learning Objectives/Skills

- Students will be able to recognize the differences between innate and adaptive immunity and the cells and signals of each.
- Students will be able to create a model for a component of the immune system and as a group, model the functions of each component in the system.
- Students will be able to work through a diagnosis of a baby with an immunodeficiency disease by a case study analysis.
- Students will perform ELISA assays and blood cell lysis experiments.
- Students will be able to read an ELISA plate and determine IgM concentration levels using a standard curve.
- Students will be able to determine phenotypes of mice by flow cytometry and graphs.
- Students will be able to explain the process of genetically engineering knockout mice.

- Students will research and report on an immunodeficiency disease.

IV. Time Requirements

This unit is based on block scheduling with 65-70minute blocks of time. The time required for the unit is 13 days minimum. It is split into two sections that can stand alone or work together in a unit.

V. Advanced Preparation

Supplies will need to be ordered weeks in advance or when your school's budget is due. Labs require materials from biological supply companies or from a nearby lab or college. See materials and equipment in section VI below. Note that on Day 1, there is a pretest to provide a way for teachers to assess their students on the curricula covered in this unit. You may want to use this pretest or create one of your own.

The following documents should be downloaded and/or printed in advance:

- *Immune System – Bozeman Science* student worksheet. (copy for Day 1)
- *Innate vs. Acquired Immunity* lecture. (download for Day 2)
- *Recognizing Antigens* student worksheet. (copy for Days 2 and 3)
- *ELISA Tutorial* student worksheet. (copy for Day 4)
- *Ice Cream Sundae ELISAs* student worksheet. (copy for Day 5)
- *MAIDS Serum IgM or IgG2 ELISA* student lab worksheet. (copy for Day 5-7)
- *Using Mice Models in Science* student worksheet. (copy for Day 8)
- *Flow Cytometry: Online Tutorial* student worksheet. (copy for Day 9)
- *Phenotyping for CD40 Knockout Mice* student lab worksheet. (copy for Day 10)
- *Assessment: Immunodeficiency Disorders* (copy for Days 11-13)

The lab preparations will take some time and it is important to read all of the information that follows under the next section. Many reagents and supplies are needed, and must be stored at the correct temperature. Some of the recipes such as 1X PBS and 1x ELISA Wash Buffer need to be made from the 10x stock solutions. It is essential that you have all the supplies before starting the lab.

VI. Materials and Equipment

Materials for this unit vary from art supplies to lab supplies that need to be ordered from a biological supply company. The materials needed will be listed below by sections.

Art supplies:

This is a list of suggested materials to use to create the cells and molecules for the immune system role-playing exercise.

- colored construction paper
- cardboard tubes

- felt
- glue and glue guns
- markers
- colored pencils
- scissors
- various types of tape (scotch, duct tape)
- pipe cleaners
- styrofoam shapes

Food/grocery:

- 3 kinds of ice cream (one should be vanilla)
- waffle bowls
- chocolate syrup
- whipped cream
- sprinkles
- paper bowls
- plastic spoons

Equipment and common lab supplies:

- computers with Internet access
- graph paper
- goggles
- gloves
- vortex
- incubator
- ice
- micropipettors (2 µl – 1000 µl)
- micropipette tips (2 µl – 1000 µl)
- liquid waste containers
- autoclave bags
- autoclave
- refrigerator
- timer
- 1 ml graduated plastic pipettes (need a full box)
- 3 ml or higher culture tubes
- 15 ml culture tubes
- test tube racks
- aluminum foil
- centrifuge
- 1.5 ml microcentrifuge tubes
- distilled water

Lab Materials and Reagents List:

ELISA LAB (Cao Lab Protocol)

Materials	Source	Product #	Storage
Serum Ig 96-well polyvinyl chloride plate	***Costar***	353921	RT
Capture Antibodies (1 mg/ml)			4°C
- Goat anti-mouse IgM	***Southern BioTech***	1020-01	
Detection Antibody (1 ml)			4°C
- Alkaline phosphatase (AP) – Goat anti-mouse Ig	***Southern BioTech***	1010-04	
Ig Standard Proteins (1 mg/ml)			4°C
- Mouse IgM	***Southern BioTech***	0101-01	
-			
Coating Buffer and Sample Diluents			4°C
- 1xPBS (*See Recipe for PBS General)			
ELISA Wash PBS			RT
- 1xELISA WASH PBS (*See Recipe for 10xELISA Wash PBS)			
- Use 10x ELISA wash buffer with Tween and add to 10L of white tap water to fill up carboy			
- pH should be 7.4			
Blocking Buffer			4°C
- 5% Bovine Serum Albumin (BSA)/PBS			
o 5 g BSA	***Sigma***	A7906	
o 100 ml 1xPBS (General; pH 7.4) **Make fresh each time*			
Detection Antibody Diluent			4°C
- 1% Bovine Serum Albumin (BSA)/PBS			
o 1 g BSA	***Sigma***	A7906	
o 100 ml 1xPBS (General; pH 7.4) **Make fresh each time*			
Phosphatase Substrate			-20°C
- 4-Nitrophenyl phosphate disodium salt hexahydrate OR			
- pNPP disodium salt hexahydrate (powder, stored in freezer -20°C) (F.W. = 371.14)	***Sigma-Aldrich***	P4744-5G	
Assay Mix			RT

- 2.65g Na_2CO_3 (F.W. = 105.39) +
- 0.1 g $MgCl_2$ (F.W. = 95.22) +
- 500 ml MilliQ water
- Adjust **pH to 9.8** with 10-15 drops HCl

Stop Solution RT

- 0.2N NaOH (F.W. = 40) + 200 ml MilliQ water
    - 1.6g NaOH in 200mL of white tap water

ELISA Wash Solution (Cao Lab Protocol)

- 10x Stock solution 1000 ml: 1000 ml 10x PBS + 5 ml Tween20 (*at 0.5% for 10x and 0.05% for 1x*)
- Shake to mix well
- Store at Room Temperature (RT)

- 10 L working solution: 1000 ml stock solution + 9000 ml MilliQ H_2O
- Fill in ELISA wash carboy, shake to mix

Phosphate Buffered Saline (PBS) – General Purpose (Cao Lab Protocol)

Stock Salt Solution Make 1000 ml **10x**

Substances	F.W.	Final con. (for **10x**)	Final con. (for **1x**)	Need (g)
Sodium chloride (NaCl)	58.44	1.369 M (8%)	0.137 M (0.8%)	80.00
Sodium phosphate dibasic anhydrous (Na2HPO4)	142.00	0.082 M (1.16%)	0.0082 M (0.116%)	11.60
Potassium chloride (KCl)	74.55	0.027 M (0.2%)	0.0027 M (0.02%)	2.00
Potassium phosphate monobasic (KH2PO4)	136.04	0.0147 M (0.2%)	0.00147 M (0.02%)	2.00

- 1000 ml – MilliQH_2O
- Stir to dissolve
- pH to 7.0-7.4
- Filter sterilize w/ 0.22 filter
- Store at Room Temperature (RT)

Prepare working solution by combining:

Make 1000 ml

- Stock salt solution 100 ml
- 900 ml MilliQ H_2O
- Store at Room Temperature (RT)

Phenotyping for Knockout Mice (Cao Lab Protocol)

5/2008

I. Preparation

1. Solutions needed

Solution	Recipe
Red Blood Cell Lysis buffer	See recipe for "Red Blood Cell Lysis buffer"
1x PBS	See recipe for "PBS for General Purpose"
Staining Buffer (SB)	1XPBS with 2% FBS and 0.09% Sodium azide (make 50 ml stock)
FC Blocker	5 ml staining buffer and 200 ml purified anti-mouse CD16/CD32 (2.4G2, 0.5 mg/ml, BD 553142)
4% Formaldehyde in 1XPBS	5.4 ml 37%Formaldehyde in 44.6 ml 1XPBS
1% Formaldehyde in 1XPBS	500 ml 4%Formaldehyde in 49.5ml 1XPBS

2. Primary antibodies and secondary reagent:

- **FITC-anti mouse CD4 (GK1.5, 0.5 mg/ml, BD 553729)**
- Biotin-anti mouse CD40 (1C10, 0.5 mg/ml, eBioscience 13-0401-85)
- Streptavidin- Phycoerythrin (PE) (SA-PE, 0.2 mg/ml, eBioscience 12-4317-87) – Make 1:50 stock with Staining Buffer.

3. **Others:**
    - **CO_2 chamber and 1 ml syringes (25G needles) for blood collection**
    - **EDTA-coated tubes (BD Vacutainer Ref # 367841)**
    - **1.7 ml eppendorf tubes**
    - **Flow tubes (12x75mm Polystyrene Tubes, Falcon#.:352054)**
    - Microcentrifuge set at 2,000 RCF (xg) and 4^{o}C

Red Blood Cell Lysing Buffer (Cao Lab Protocol)

RBC Lysing Buffer Make 200 ml

Substances	F.W.	Final con.	Need (g)
Ammonium Chloride (NH4Cl)	53.49	0.155 M (0.829%)	1.658
Potassium Bicarbonate (KHCO3)	100.12	0.01 M (0.1%)	0.2
Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (Disodium EDTA)	372.24	0.1 mM (0.00372%)	0.0074

- 200 ml – MilliQ H_2O
- Stir to dissolve
- pH to 7.2-7.4
- Filter sterilize w/ 0.22 filter
- Refrigerate

VII. Student Prior Knowledge and Skills

Students should have prior knowledge in cell biology and genetics before starting this unit. Students should have experience using a vortex, micropipettors, centrifuge, and sterile techniques.

VIII. Daily Unit Plans

SECTION 1: THE IMMUNE SYSTEM

Day 1:

1. ***Pretest*** to assess prior knowledge of immune system concepts. This is optional. An example of a pretest is included in this curriculum or you can create your own to fit your classroom.

2. ***Overview video –*** "Immune System" – Bozeman Science http://www.bozemanscience.com/immune-system with guided worksheet. Distribute the worksheet to each student and either collect at the end of the video or engage in a class discussion on the video content.

3. ***Homework Reading*** **-** Assign textbook reading Chapter 35 Urry,L.A., Cain, M.L., Wasserman, S.A., P.V., Jackson, R.B., Reece, J.B. (2014). *Campbell Biology in Focus, AP Edition.* Glenview, IL: Pearson Education, Inc. Ask students to outline the chapter or take notes as they read. Optional work can include answering section review questions and end of chapter questions.

Day 2:

1. ***Lecture*** **-** The Immune System: Innate vs. Acquired Immunity. Download before class and use as PowerPoint lecture or show through Google Slides.
2. ***"Recognizing Antigens" Part 1***– Role-playing exercise to allow students to act out the immune response as T and B cells recognize antigens. Part 1 will consist of students choosing cells to represent (macrophages, B cells, T cells, antibodies, killer T cells, and MHC2). They will then create their surface proteins needed for recognition and communication between the cells. Hand out the worksheet for students to see instructions and a list of cells or molecules to choose from. Art supplies will be needed for this activity or it can be assigned as homework to save class time.

Day 3:

1. ***"Recognizing Antigens" continued– Part 2***. Students complete activity by acting out immune response by B and T cells to antigens.

Day 4:

1. ***Case Study:*** Students will read a case study on a patient with an immunodeficiency disease adapted from the case study collection at the "National Center for Case Study Teaching In Science." I used "The Case of Baby Joe" for my class. You can choose any of the case studies you prefer but in order to access the answer key, you must pay for a subscription.

2. ***ELISA tutorial:*** Hand out the student worksheet and ask students to complete for homework. The assignment can be graded or used in a class discussion the next day. Tell students that the ELISA they will be doing is a little different and they should study the Sandwich ELISA diagram found at http://www.abcam.com/ps/pdf/protocols/Sandwich_Elisa_diagram.pdf.

3. ***Next Day prep*** **-**Be sure to have purchased the food items for the ELISA sundaes on Day 5.

4. ***Lab prep-*** Gather and prepare all materials for Day 1 of the ELISA lab – coating the plates. (See Teacher Guide for lab.)

Day 5:

1. ***Ice Cream Sundae ELISAs*** **–** Students will make ice cream sundaes while discussing the ELISA protocol. Purchase items ahead of today's class.

a. Ask students to take a paper bowl, add a waffle bowl to it, and obtain one spoon. Explain to students that the waffle cone represents the capture antibody they will add to their microplate today.

b. Instruct students to choose one flavor of ice cream and add it to their waffle bowl. Ask students what they think the ice cream represents. Additional question could be to ask students what an antibody attaches to. Students should say that the ice cream represents samples from patients that contain the target protein or antigen that will bind to the capture antibody.

c. Instruct all students EXCEPT those with vanilla ice cream to add chocolate syrup to their ice cream.

d. Ask students what the syrup may represent (detection Ab).

e. Ask them why the students with vanilla ice cream were not allowed to add syrup. They should realize that the students with the vanilla ice cream have the negative plate or control plate. The detection Ab and other components added will be washed away because the sample (vanilla ice cream) did not contain the target protein.

f. Instruct students to add whipped cream to those with ice cream other than vanilla. This will represent the secondary Ab with an enzyme attached.

g. Now ask students to add sprinkles to the whipped cream. This represents the substrate and color change for positive plates.

h. Try to challenge the students by telling them that one of the flavors of the positive samples is really negative for the target protein. Ask them why it still changed colors? Students should answer that it could be a "false positive" result.

2. ELISA pre-lab –Complete steps 1-4 of Day 1 of the **–** "MAIDS Serum IgM or IgG2 ELISA" lab. Students will be coating the ELISA plates with the capture antibody and incubating overnight.

3. ***Next day lab prep*** **-**Prepare all materials and supplies for Day 2 of the "MAIDS Serum IgM or IgG2 ELISA" lab. **NOTE: due to time restraints I completed the first part of Day 2 (steps 7-13) of the lab in the morning before the students came to class. They had just enough time to prepare samples and standards and add them to the plates during that block of class time.**

Day 6:

1. ***ELISA lab –*** "MAIDS Serum IgM or IgG2 ELISA". (Adapted from Cao lab protocol.) Serum samples will be used from non-infected mice and infected mice to find IgM levels. If you have not done serial dilutions in class before, be sure to go over the directions carefully and remind students to follow each step as it is important to dilute the samples correctly.

2. ***Next day lab prep*****-**Prepare materials and supplies for Day 3 of the lab. **NOTE: due to time restraints and the 2 hour incubation period, I completed the wash steps and added the detection Ab (steps 23-26) in the morning before the students come to class.**

Day 7:

1. ***ELISA lab data analysis and results*** **-** students use the standard curve to estimate concentration levels along with actual data collected from the Cao Lab. The color change usually takes 15-20 minutes but I found it took 1.5 hours instead in my classroom so if the color doesn't change right away, leave in a dark place like a drawer for several hours in case it is taking longer than usual.

SECTION 2: GENETIC ENGINEERING AND ANIMAL MODELS

DAY 8:

1. ***Video:*** Ted Talk on using animal models in the lab. Steve Ramirez and Xu Liu: "A Mouse. A laser Beam, A manipulated memory".

2. ***Reading***: "Knockout Mice" and "Transgenic Mice" with guided worksheet.

Day 9:

1. ***Flow Cytometry Tutorial*** with video worksheet. Check access to the following sites.

"Molecular Probes Tutorial Series – Introduction to Flow Cytometry"

and "Molecular Probes Tutorial Series – Analyzing Flow Cytometry Data" .

2. ***Next Day Lab prep***-Depending on whether you are able to do this lab at a nearby research facility, you will want to gather supplies that the lab may expect you to bring with you.

Day 10:

1. ***Lab at the Biomedical Research lab at the University of New England –*** Students take a field trip to the research lab and perform lab "Phenotyping for CD40 Knockout Mice." Students will prepare samples by performing red blood lysis and then run the Flow cytometer to identify wild type and knockout mice.

SECTION 3: IMMUNODEFICIENCY DISORDERS

DAYS 11-13: *Final Assessment: Research project and Presentation*

Essential question: What types of genetic mutations in the immune system cause immunodeficiency disorders? What cells are missing or defective? What are the consequences of this mutation?

Project Descriptor: Students will:

•Choose an immunodeficiency disorder to research.

•Give a general description of the disease and symptoms.

•Identify and explain the mutation that causes the disruption of the immune system.

•What are the consequences of this mutation?

•Describe any treatments or therapies that are available.

•Cite all sources used to find the information.

•Choose a creative way to display your research (poster, slide presentation, movie, story, etc.)

•Present your findings to the class.

Sample Grading Rubric

	Excellent (4 points)	**Proficient (3 points)**	**Needs Improvement (2 points)**	**Below Expectations (1 point)**
Description of disease and symptoms	A full description of what causes the disease AND a complete list of symptoms is present	A full description of what causes the disease AND a partial list of symptoms is present	A partial description of what causes the disease AND a partial list of symptoms is present	Either a description of the disease or the list of symptoms is missing.
Identification and explanation of mutation	The type of mutation and name of mutation is present AND how the mutation causes the immune system to malfunction is present.	The name of the mutation is present but the type of mutation is missing AND how the mutation causes the immune system to malfunction is present.	Either the name and type of mutation is present OR the explanation of the mutation but not both.	The identification of the mutation AND how it disrupts the immune system are missing.
Consequences of the mutation	A complete description of the end result of the mutation on the person's life expectancy AND quality of life is present	A partial description of the end result of the mutation on the person's life expectancy AND quality of life is present	The life expectancy of the person is present but NOT how the quality of life is affected by the mutation.	The consequences of the mutation on the person's life are missing.
Treatments and Therapies	A complete list of treatments and therapies are present with an example of how they help the person is present.	A partial list of treatments and therapies are present with an example of how they help the person is present.	A complete list of treatments and therapies are present but NO examples of how they help the person are present.	Treatments and therapies are missing.
Creative display of information	The presentation is displayed in a creative way and organized so that the information flows in a way that makes sense to the audience.	The presentation is displayed in a creative way, BUT is NOT organized so that the information flows in a way that makes sense to the audience.	The presentation is displayed but not in a creative way AND not organized.	The presentation is missing.

VIII. ELISA Teacher Guide: Pre-lab Preparations

The ELISA lab takes 3 days to complete and time before each class to prepare materials. A list by day is given to guide teachers as to what to prepare for lab groups.

DAY 1: Plate Preparation

The day before the lab, prepare the 10x PBS stock solution per the recipe on page 10. Then prepare the 1x PBS buffer from the 10x PBS stock solution by adding 100 ml of 10x PBS to 900 ml of distilled water. Store at RT.
For each lab, group of 2 or 4 students, gather one 96-well plate, 40 ml 1xPBS, one 15 ml culture tube, 10 ml graduated cylinder, a 10 µl – 100 µl micropipettor, pipette tips, 80 µl of Capture Ab, waste beaker, gloves, a plate cover, and an autoclave bag.

DAY 2: ELISA lab

The day before the lab or that morning, prepare the 10x ELISA Wash Buffer per the recipe on page 10. Then prepare the 1x ELISA Wash Buffer by adding 100 ml of 10x ELISA Wash Buffer to 900 ml of distilled water. Distribute 235 ml 1x ELISA Wash Buffer per lab group.
If you don't have a multichannel pipette, you will need to have a lot of 1 ml pipettes to complete the wash steps. The lab is written with 1ml pipettes, but a multichannel pipette is much faster.
Prepare the 5% BSA/PBS and distribute 17 ml per group. Store at 4^{o}C until use.
Each lab group will need a multichannel pipette, pipette tips, 1 xELISA Wash Buffer, liquid waste beaker, 80 ml distilled water, waste beaker, stack of paper towels, 17 ml 5% BSA/PBS, plate cover, 37^{o}C incubator, ice bath, 2 µl Mouse IgM standard, 5 µl of each serum sample, 2 µl-20 µl micropipettor, 100 µl to 1000 µl micropipettor, 10 µl to 100 µl micropipettor 12- 3ml or higher test tubes, 1x PBS, vortex, marker, goggles, gloves, 1.4-1.5 ml microcentrifuge tubes, and refrigerator.

DAY 3: ELISA LAB

The day before the lab or that morning, prepare the 1% BSA/PBS and distribute 10 ml to each group. Store at 4^{o}C until use. Prepare the Assay Mix and distribute 10 ml per lab group.
Each lab group will need a multichannel pipette, pipette tips, 1 xELISA Wash Buffer, liquid waste beaker, 80 ml distilled water, waste beaker, stack of paper towels, 10 ml 1% BSA/PBS, plate cover, 10mg PNPP, 10 ml Assay mix, 5 ml 0.2N NaOH, 2 µl-20 µl micropipettor, 100 µl to 1000 µl micropipettor, 10 µl to 100 µl micropipettor, 2 µl Detection Ab, 37^{o}C incubator ,vortex, graph paper, marker, goggles, and gloves.

XI. Student Section

A.

Pretest

Name: _____ Class: _____ Date: _____ ID: A

AP Biology **Immune System Pretest**

Multiple Choice
Identify the choice that best completes the statement or answers the question.

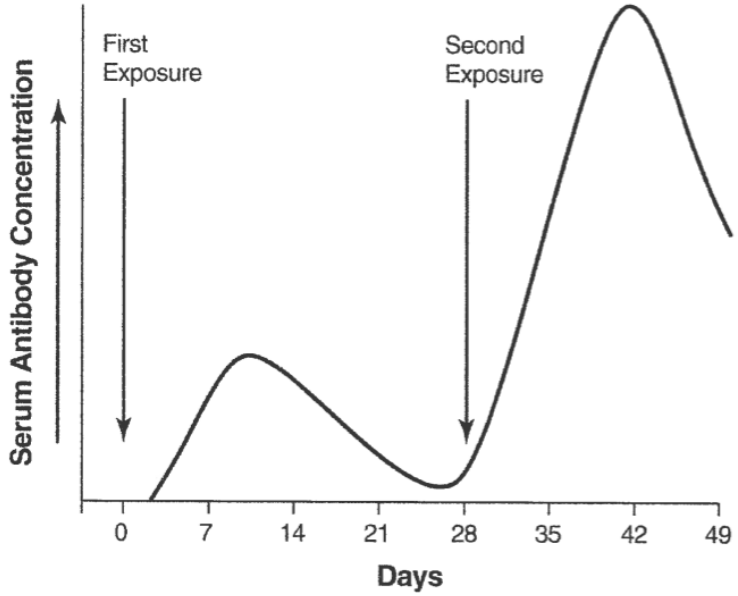


Figure 37-2

____ 1. Examine Figure 37-2. Which type of immunity is the second (right) peak in the graph illustrating?
a. active immunity
b. passive immunity
c. nonspecific immunity
d. immune system failure

____ 2. Examine Figure 37-2. How does the second response to the antigen differ from the first response?
a. In the second response, fewer T cells are activated.
b. The first response is faster than the second response.
c. In the second response, a greater number of B cells are activated.
d. The first response produces more antibodies than the second response.

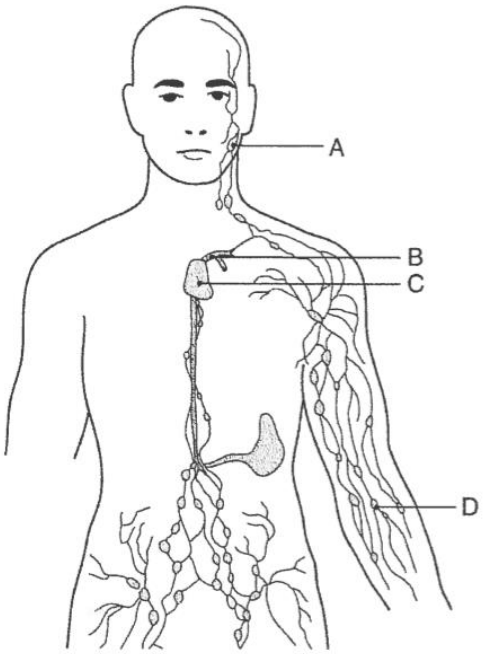


Figure 37-3

___ 3. Examine Figure 37-3. Which structure has a role in activating T cells?
a. Structure A
b. Structure B
c. Structure C
d. Structure D

___ 4. Which of the following cells are involved in nonspecific immunity?
a. B cells
b. cytotoxic T cell
c. helper T cell
d. neutrophils

___ 5. An Rh^- mother gives birth to an Rh^+ child. In the process, some of the child's blood mixes with the mother's blood. The mother develops antibodies against the Rh antigen. A second pregnancy ends in a miscarriage. What type of immunity is this an example of?
a. active immunity
b. interferon immunity
c. nonspecific immunity
d. passive immunity

___ 6. The inflammatory response can cause
a. permanent immunity.
b. pain, swelling, and fever.
c. antibodies to bind to antigens.
d. killer T cells to attack infected cells.

____ 7. The body's most important nonspecific defense is
a. the skin.
b. cell-mediated immunity.
c. the inflammatory response.
d. permanent immunity.

____ 8. When a person receives a vaccine, his or her body
a. receives antibodies against a specific pathogen.
b. creates plasma cells that can produce antibodies against the specific pathogen.
c. creates antigens to fight the specific pathogen.
d. immediately begins fighting the infection caused by the pathogens.

____ 9. Humoral immunity is carried out by
a. killer T cells.
b. lymphocytes.
c. antibodies.
d. macrophages.

____ 10. A person who has received a vaccine against polio
a. is able to produce antibodies against polio.
b. is more susceptible to the polio virus than someone who has not had the vaccine.
c. has polio antibodies in the bloodstream.
d. has antipolio killer T cells in the bloodstream.

____ 11. An immune response is triggered by a(an)
a. antibiotic.
b. antibody.
c. antigen.
d. histamine.

____ 12. Asthma is an example of
a. the immune system attacking its own body cells.
b. the immune system overreacting to an antigen.
c. an autoimmune disease.
d. an infection.

____ 13. An example of an autoimmune disease is
a. asthma.
b. allergies.
c. multiple sclerosis.
d. strep throat.

____ 14. Autoimmune diseases result when the immune system
a. fails to distinguish self from nonself.
b. overreacts to certain antigens.
c. is weakened by asthma.
d. all of the above

____ 15. HIV weakens the immune system by killing
a. antibodies.
b. B cells.
c. helper T cells.
d. killer T cells.

Other

USING SCIENCE SKILLS

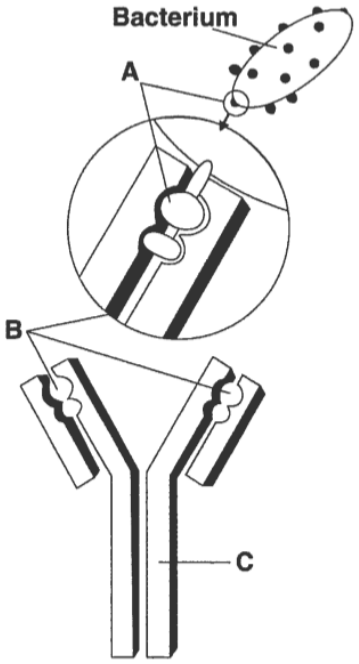


Figure 40-2

1. **Interpreting Graphics** What does Figure 40-2 represent?
2. **Interpreting Graphics** What does letter B represent?
3. **Interpreting Graphics** Which structure or structures trigger the production of antibodies?
4. **Interpreting Graphics** What kind of immunity is represented in Figure 40-2?
5. **Interpreting Graphics** What role do antigen-binding sites play in an infection?

B.

Name _____

Bozeman Science - Immune System

http://www.bozemanscience.com/immune-system

Paul Anderson starts his video with an analogy of the immune system being like a castle.

1. The castle wall is like our _____ that provides us with a _____. It also has a _____ _____ that makes it hard for bacteria to live there.
2. Chemicals on the surface or our skin and bacteria can also provide protection against _____.
3. Occasionally, you get a cut and it is equivalent to when the wall is breached.
4. Our bodies respond by _____.
5. Our soldiers are the _____or big eaters. The invaders are called _____. Something to remember is that macrophages attack _____ invader.
6. The specific immune response is more like the _____. The antigens have specific _____ on their surface.
7. We have _____ that recognize antigens.
8. Antibodies are Y-shaped. At the top are two arms with different _____ that can dock to the _____ surface. This _____ the antigen so the macrophages can find them.

Specific Immunity

1. Lymphocytes are a type of _____. Two types: B and T lymphocytes.
2. **B lymphocytes** are made in the _____ and are responsible for a _____ response found in the fluids of your body.
3. B lymphocytes produce _____. A näive B cell senses the _____ of an antigen and produces antibodies that are specific to the antigen surface.
4. Then memory cells are produced.
5. **T lymphocytes** are responsible for _____ - mediated response that targets infected cells.
6. T lymphocytes are made in the _____ and produce killer T cells. These dock to infected cells and kill our _____ cells.

Flow chart

Begin with an antigen that is has been eaten by a macrophage that chops it up into pieces. Pieces of the antigen are presented on the surface by _____ chemical and then a T-_____ cell (CD4) docks to the antigen and initiates both the _____ and _____ responses to produce B cells, antibodies, activate macrophages, and killer T cells.

Patients with _____ don't have a normal immune response because the virus infects the _____.

C.

Recognizing Antigens

The immune system is acomplex system with specialized cells and structures interacting together. One event, such as a simple cut to your finger, can cause a chain reaction of responses. Each component has an important function, so it is essential to look at the system as a whole. This activity will be an exercise in role-playing the immune system response that occurs after a person receives a cut on the arm. It will consist of choosing a component of the immune system, creating a costume or prop to represent the component, and then working as a class to act out the response of the immune system.

Day 1:

Choose one of the following to represent.

- Antibodies
- Antigen
- Antigen-presenting cell
- Arm
- B cells
- Class I MHC molecule
- Class II MHC molecule
- Cytotoxic T cells
- Helper T cells
- Interleukin-1
- Interleukin-2
- Killer T cells
- Macrophages
- Memory B cells
- Memory T cell

Use the material provided to create props or costumes to represent your part. Be sure to speak with others in the class if you will be directly connected to another person at some time during the role-playing exercise.

Day 2:

You will have 30 minutes to work on practicing the play. Then you will act out the immune response for your teacher or other classes.

D.

Name _____

ELISA Tutorial

A common tool used by scientists and researchers to test for the presence of an antibody or antigen is the enzyme-linked immunosorbent assay (ELISA). In fact, ELISA is used in home pregnancy and HIV tests. To understand how the test works, go to the *ELISA activity* on the Biology Project website and answer the following questions.

1. There are four main components to an ELISA. List each one below.
   _____
   _____
   _____
   _____

   Start the "Positive ELISA animation". (It moves quickly so you will need to stop it at times to answer the questions)

2. For an HIV test, what is the first component added to the ELISA plate?
   _____

3. What is added next? _____

4. The next step is not clearly shown but involves washing the plate with buffers. What is the scientist trying to wash away?
   _____
   _____
   _____

5. What is the third component added? _____

6. After the second wash is complete, what components are left in the plate? How are they connected?
   _____
   _____
   _____

7. The last step is to add a substrate. What are we looking for if the substrate reacts with the secondary antibody? _____

8. Watch the "Negative ELISA test" animation. Explain what happens that causes the negative result (no color) to appear.

9. What is meant by a "false positive"?
   _____
   _____
   _____

10. Finish the tutorial by looking at the results of the 3 patients tested. Continue to the short 4 question quiz at the end.

E.

Name_____

Ice Cream Sundae ELISA

The protocol for an ELISA lab can be complicated due to the amount of pipetting involved and the multiple washes that take place. To give you a simpler description of what the ELISA protocol involves, we will be making ice cream sundaes while discussing the components used in the ELISA procedure.

Instructions:

1. Obtain a paper bowl, a waffle bowl, and a spoon.
2. The paper bowl represents the microplate well that you will be adding your sample and components to during the ELISA assay. What do you think the waffle bowl represents? _____
3. Add one scoop of ice cream of your choice. What flavor did you choose? _____
4. If you start with an antibody, what do you think the ice cream must represent? (Hint: what binds to an antibody?) _____
5. If you chose vanilla ice cream, please stop the activity at this time. If you chose a different flavor of ice cream, go to step 6.
6. Add chocolate syrup to your ice cream sundae. What component would the syrup represent? _____
7. Can you guess why the students with vanilla ice cream were not allowed to add chocolate syrup? (Hint: what do you always need to have for each experiment you perform in the lab?) _____
8. Add whipped cream to your sundae. This is the substrate.
9. Add sprinkles to your sundae if you would like. This represents a positive result. Why are we adding sprinkles to the whipped cream (substrate)? _____
   ___What would you expect to happen in the experiment?
   _____
   _____
10. Some experiments result in a "false positive". Can you explain how we can show a "false positive" in our sundaes? _____
    _____
    _____

F.

Name_____

MAIDS Serum IgM ELISA

Objective:

To determine the estimated total concentration of IgM in a mouse serum solution.

Learning Targets:

- Students will be able to perform an ELISA
- Students will perform serial dilutions.
- Students will plot a standard curve and plot data from UNE lab.

Materials List for 1 plate (group of 2 or 4)

96-well microplate
80 µl Capture Antibody
40 ml 1x PBS
plate cover
235 ml 1x ELISA wash buffer
80 ml distilled water
17 ml 5% BSA/PBS
2 µl Mouse IgM standard
5 µl of each serum sample
2 µl of Detection Antibody
10 ml 1% BSA/PBS
10mg PNPP Substrate soln
10 ml Assay Mix
5 ml 0.2N NaOH
graph paper
sample data from lab
Goggles
14- 1.5ml microcentrifuge tubes
10 µl-100 µl micropipettor
2 µl-20 µl micropipettor
100 µl to 1000 µl micropipettor
pipette tips
ice bath
100 µl to 1000 µl multichannel pipette
waste beaker for liquid
incubator
12- 3ml or higher test tubes
vortex
refrigerator
3- 15ml culture tubes
markers
paper towels
timer
Waste beaker for pipettes and tips
gloves

Safety Reminders:

- Avoid mixing solutions together.
- Wear gloves at all times.
- Goggles should be worn on Days 2 and 3.

Procedure

DAY 1:

Plate Preparation

1. Prepare plate layout sheet for each the group. Use the "96-well plate layout" form below to record both standards and samples. Each group of 4 students will prepare for two rows of standards and two rows of samples.

	1	2	3	4	5	6	7	8	9	10	11	12
STD												
STD												
STD												
STD												

2. Dilute the Capture Antibody by adding 80µl of Capture Antibody into 10ml of Coating Buffer (1x PBS) for each plate.
3. Coat the plate by adding 100 µl of the diluted Capture Antibody into each well of the 90-well microplate. Gently tap the plates on each side to make sure the bottom of each well is evenly covered. Cover plate with a piece of cardboard or the top of one of the plastic pipette tip boxes. Incubate plate overnight at RT.
4. Prepare tubes for both samples and standards.

Day 2

7. Using a plastic 1ml pipette, remove any liquid left in the wells of the plate.
8. **Wash plate** by using a micropipette and adding 200 µl of 1x ELISA wash buffer to each well.
9. With a plastic 1ml pipette, remove the liquid in each well and dispose into the "liquid waste" container at your lab bench.
10. **Repeat steps 8 and 9 two more times for a total of three washes.**
11. Wash one more time with 200 µl **distilled water.** Then remove liquid with a plastic 1ml pipette and dispose in the liquid waste container.
12. After the last wash with distilled water, pat the plate dry on a stack of paper towels until most of the liquid has been disposed of.
13. **Block Plate** by adding 175 µl 5% BSA/PBS to each well. Gently tap the plates on each side to make sure the bottom of each well is evenly covered. Cover plate with a piece of cardboard or the top of one of the plastic pipette tip boxes. Incubate plate at 37^{o}C for 1 hour.
14. During this incubation period, prepare the samples and standards (KEEP EVERYTHING ON ICE). For **each** group of 4 students, **one** set of standards should be made and **one** set of each sample. (Suggestion: one student adds all the 1xPBS to the tubes and other students are responsible for 1 sample or the standards).

<u>Preparation of Standards according to chart below:</u>

Standard	ng/ml	Mouse IgM	Diluent (1xPBS)
1	1000	2 µl	2 ml – 2 µl
2	500	1 ml from std 1	1 ml
3	250	1 ml from std 2	1 ml
4	125	1 ml from std 3	1 ml
5	62.5	1 ml from std 4	1 ml
6	31.25	1 ml from std 5	1 ml
7	15.625	1 ml from std 6	1 ml
8	7.813	1 ml from std 7	1 ml
9	3.906	1 ml from std 8	1 ml
10	1.953	1 ml from std 9	1 ml
11	0.9765	1 ml from std 10	1 ml
12	0	none	1 ml

a. Label a series of 12 tubes as S1, S2, S3, S4, S5, S6,S7, S8, S9, S10, S11, and S12.
b. Add 2 ml of 1xPBS into the S1 tube.
c. Remove 2 µl of 1xPBS from the S1 tube and add 2 µl of the IgM standard stock solution (stock concentration is 1 mg/ml) into tube S1. Vortex to mix.
d. Add 1 ml 1x PBS to tubes S2-S12.
e. For each standard, take 1 ml out of the S1 tube and add it to the S2 tube, vortex to mix. As in the table above, take 1 ml out of the S2 tube and add it to the S3 tube, vortex to mix. Continue this procedure through tube S11. **Do not add anything from S11 to S12.** (S12 is the "0" concentration while the S1 has the highest protein concentration).

Preparation of Samples according to chart below:

Tube #	Dilution	Sample	Diluent (1xPBS)
T0	1:20	5 µl	95 µl
T1	1:400	40 µl from T0	760 µl
T2	1:1200	300 µl from T1	600 µl
T3	1:3600	300 µl from T2	600 µl
T4	1:10800	300 µl from T3	600 µl
T5	1:32400	300 µl from T4	600 µl
T6	1:97200	300 µl from T5	600 µl

FOR EACH SAMPLE:

a. Label a series of 7 tubes T0, T1, T2, T3, T4, T5, and T6.
b. Add 95 µl of 1xPBS to tube T0.
c. Add 5 µl of sample to tube T0. Vortex to mix.
d. Add 760 µl of 1xPBS to tube T1.
e. Take 40 µl out of tube T0 and add into tube T1. Vortex to mix.
f. Add 600 µl of 1xPBS to tubes T2-T6.
g. Make 1:3 serial dilutions by taking 300 µl from tube T1 and adding into Tube T2. Vortex to mix. Continue this procedure through tubes T3-T6 according to the chart above.

15. Using a plastic 1ml pipette, remove any liquid left in the wells of the plate.
16. **Wash plate** by using a micropipette and adding 200 µl of 1x ELISA wash buffer to each well. **Switch tips after each row across.**
17. With a plastic 1ml pipette, remove the liquid in each well and dispose into the "liquid waste" container at your lab bench. **Switch pipettes after each row across.**
18. **Repeat steps 16 and 17 two more times for a total of three washes.**
19. Wash one more time with 200 µl **distilled water.** Then remove liquid with a plastic 1ml pipette and dispose in the liquid waste container. Pat the plate dry on a stack of paper towels until most of the liquid has been disposed of.
20. Add 100 µl of each standard to the appropriate wells you marked in the table in step 1 on Day 1. **Note:** you are putting the same standard in 2 rows at the top and also in the middle of the plate so can use the same tip but be sure to switch tips each time you change standard dilutions.
21. Add 100 µl of each sample dilution from tubes T1 to T6 to the appropriate wells you marked in the table in step 1 on Day 1. Be sure to switch tips for each sample tube.
22. Incubate plates overnight at 4°C.

DAY 3:

23. Obtain your well plate from the refrigerator.
24. Wash plates three times with ELISA wash and once with distilled water **as in steps 15-19 on Day 2.**
25. Mix 2 µl of Detection Antibody with 10 ml 1% BSA/PBS per plate.
26. Add 100 µl of the diluted antibody to each well. Gently tap the plates on each side to make sure the bottom of each well is evenly covered. Cover plate with a piece of cardboard or the top of one of the plastic pipette tip boxes. Incubate plate at 37^{o}C for 2 hours.
27. Prepare PNPP Substrate during the last **5-10 minutes of incubation.** For each plate, mix 10 mg (0.01g) of PNPP with 10 ml of assay mix (final concentration of 1 mg/ml).
28. Remove liquid and wash plates three times with ELISA wash and once with distilled water **as in steps 15-19 on Day 2.**
29. Add 100 µl of PNPP Substrate Solution to each well. Start at the right side of the plate where the most diluted samples are and work backwards in each row
30. Cover the plate and incubate at RT **in the dark** for 15 minutes or until color changes. Once you see the color change, move to the next step.
31. Add 50 µl of 0.2N NaOH Stop Solution to each well. Start at the right side of the plate where the most diluted samples are and work backwards in each row
32. Looking at the results of the color change on your plate, match the color from the T3 dilution tube (1:3600) to a corresponding color of the standard. Record your results in the table below.

Sample ID	Standard # color matches to at 1:3600 dilution of sample	Ng/ml in standard tube	Estimated concentration of IgM in sample

Analysis Questions:

1. Looking at your data, do you believe one of your samples comes from a mouse infected by the mouse type of AIDS (MAIDS)? Why?

_____
_____
_____
_____

2. Summarize what happens in the well of the plate during an ELISA test. Include all components that would be found in a positive sample.

_____
_____
_____
_____
_____
_____
_____

3. The experiment requires a standard curve to be used to find the concentration of IgM in the mouse serum sample. Using the following data, plot the standard curve on graph paper.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Conc. ng/ml	1000	500	250	125	62.5	31.3	15.6	7.8	3.9	2	1	0
405 absorbance	0.9	0.7	0.5	0.4	0.3	0.2	0.15	0.1	0.5	0.3	0.1	0

4. Find the concentration of IgM for the following 405 absorbance numbers for the following samples using the standard curve you graphed.

a. 0.64 Concentration _____
b. 0.34 Concentration _____
c. 0.85 Concentration _____

G.

Name_____

Using Mice Models in Science

Part 1: "Knockout Mice"

Go to the NIH website and read "Knockout Mice" . Answer the following questions from each section.

What is a knockout mouse?

1. How is a knockout mouse made?

_____
_____
_____
_____

2. How does the mouse change when the genes are manipulated?

_____
_____
_____
_____

What are knockout mice used for?

3. Why are mice used to study human genes? Give two examples.

_____
_____
_____
_____

4. How are mouse models named? Give an example of each.

_____
_____
_____
_____
_____
_____

What are the drawbacks of knockout mice?

5. Explain the two limitations that present themselves in knockout mice.

How are knockout mice made?

6. Why do researchers use embryonic stem cells to create the knockout mice?

7. What is meant by "in vitro"?

8. The first strategy discussed is gene targeting or homologous recombination. Go to Nobelprize.org and read through the procedure. Summarize this strategy in your own words.

9. How is gene trapping different from gene targeting?

10. What are homozygous knockouts?

_____
_____
_____
_____

Which production method is best?

11. Looking at the advantages and disadvantages of each strategy, which do you believe is better and why?

_____
_____
_____
_____
_____
_____

Part 2: "Transgenic Mice"

Go to the Learn Genetics website and read the information on transgenic mice. Then answer the following questions.

1. Who won the Nobel Prize in 2007? What field of study was the research in?

   _____
   _____
   _____

2. What question did he raise regarding the future of medicine?

   _____
   _____
   _____

3. What do homeotic genes code for?

   _____
   _____
   _____

4. Create a flow chart below showing the steps to making a knockout mouse.

H.

Name_____

Flow Cytometry
Online Tutorial Worksheet

Introduction to Flow Cytometry

As you watch the tutorial *Molecular Probes Tutorial Series – Introduction to Flow Cytometry,* answer the following questions.

1. What does the flow cytometer use to count cells and analyze them?

_____

2. What can the flow cytometer tell a scientist about a cell?

_____

_____

3. What is important about the way the cells are transferred into the interrogation point?

_____

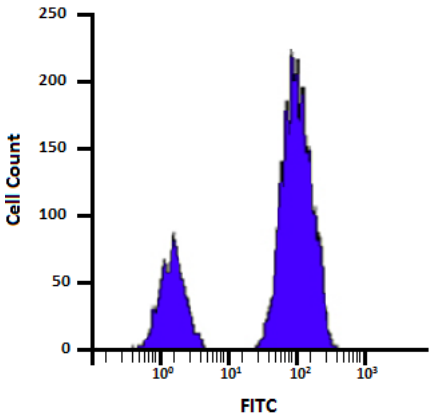
_____

4. How big are the cells that the flow can detect?

_____

5. When cells go through the laser and present a forward scatter pattern, it can determine the _____ of the cell.

6. The scattered light received by the detector will send data to a computer. This can show the size of cells. Explain from this histogram if the majority of cells in the sample are large or small. How do you know?



http://aws.labome.com/figure/te-125-9.png

_____

_____

_____

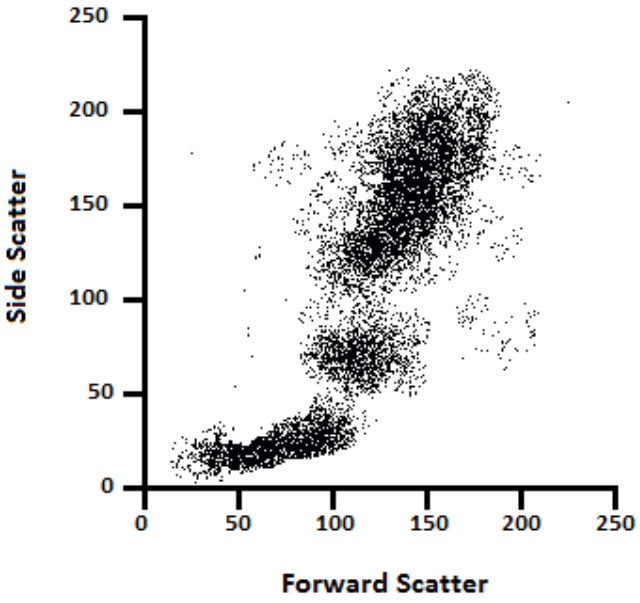
7. When light is scattered at larger angles and off to the side, it is called a side scatter. What causes this side scatter?

_____

_____

_____

8. Plotting a two dimensional scatter plot can tell you more than a one dimensional histogram. Label the 2D scatter plot below with the types of cells you would expect in a blood sample.



_____

_____

_____

http://aws.labome.com/figure/te-125-10.png

9. Fluorescence occurs when fluorophore are _____ and then return to their _____ _____. The energy in the emitted light is dependent on the energy level that it is excited and it relates directly to _____ and _____.

10. Studying specific cells can be done using a fluorophore-labeled _____ that will bond to specific cells and reading data collected by the flow cytometer. Fluorescence data is collected by the amount of light detected.

11. Why is a threshold important?

_____

_____

_____
_____
_____

Analyzing Flow Cytometry Data
Watch the tutorial *Molecular Probes Tutorial Series – Analyzing Flow Cytometry Data*.

1. As cells pass through the laser, the light pulse is converted into a _____ pulse that is then converted into a _____ value.

2. There are 3 ways to measure the pulse. What are they and what can they tell you?
   1) _____
   2) _____
   3) _____

3. What two types of graphs are used?

_____

4. In our upcoming field trip to the UNE lab, you will be detecting whether you have cells from a wild-type mouse or one that is a CD4 Knockout mouse. Based on the tutorial, would you use a linear or log scaling? Why?

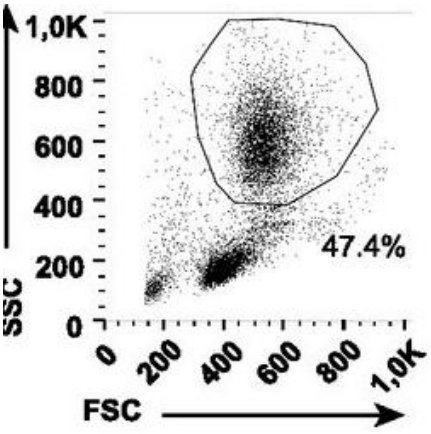
_____
_____
_____
_____

5. What does gating allow you to do?

_____
_____

6. In the plot below, which cell type population are we gating?

_____



http://www.bloodjournal.org/content/bloodjournal/126/17/2016/F5.large.jpg?sso-checked=true

7. How is compensation calculated for the two color samples?

_____

_____

STOP VIDEO AT 9:50.

I.

Name_____

Phenotyping for Knockout Mice

As a group, decide how you will handle sample tubes and control tubes. For example, you can divide tubes between students.

Materials needed per group of two students

2.5mL of Red Blood Cell Lysis Buffer on ice
15mL of 1X PBS buffer on ice
150µL FC Blocker on ice
2.5µl Primary Antibody for CD40, Biotin-anti-mouse CD40
150µl of 1% formaldehyde PBS on ice
60µl SA-PE (1:50)
60µl of Staining Buffer (SB)
16 - 1mL plastic pipettes to remove supernatant
Micropipettors (need range from 0.5ul to 1ml)
Tips
Test tube racks
Ice in beaker or container
Timer
2 Waste beakers (one for pipette tips, one for liquid waste)
Foil
Gloves
Goggles

Shared Materials:

Vortex
Centrifuge
Refrigerator

Procedure

(Per group of 2 students)

1. Obtain a 75µl sample of blood for each student from the instructor (one is sample and one is non-stained control).
2. Record the sample ID number from the tube. _____

*****From this step forward, all tubes containing cells should be kept on ice! *****

3. Add 1mL Red Blood Cell Lysis buffer to each tube. Vortex to mix.
5. Incubate on ice for 3 minutes.
6. Vortex tubes again and incubate on ice for another 2 minutes.
7. Place tubes in centrifuge for 5 minutes. Run at 2000 RCF at 4^{o} C. Return tubes back to ice.
8. Using a **new** plastic pipette, remove as much supernatant as possible from each tube **without disturbing the pellet**. <u>If you disturb the pellet, place in centrifuge for another 2 minutes to</u>

obtain the pellet again. A 250µl micropipette can be used if the plastic pipette is not sufficient for removing all liquid around the pellet). Place liquid in waste beaker. Return tubes back to ice.
9. First Wash: Add 1mL of 1X PBS in each tube and resuspend the pellet by pipetting up and down several times to mix. Use a new pipette for each tube. Vortex. Return tube back to ice.
10. Centrifuge samples for 5 minutes as in Step 7.
11. Remove the supernatant from each tube using a new plastic pipette for each tube. Place liquid in waste beaker. Return tubes back to ice.
12. Second Wash: Add 1mL of 1X PBS in each tube and resuspend the pellet by pipetting up and down several times to mix. Use a new pipette for each tube. Vortex. Return tube back to ice.
13. Centrifuge samples for 5 minutes as in Step 7.
14. Remove the supernatant from each tube using a new pipette. Place liquid in waste beaker. Return tubes back to ice.
15. Using a micropipettor, add 50ul FC Blocker to each tube and resuspend the pellet by pipetting up and down several times to mix. Switch tips between tubes. Vortex to mix.
16. Incubate samples on ice for 30 minutes.
17. To the sample tube (**not the "non-stained" control tube),** add 2µl Biotin-anti-mouse CD40 per tube directly into the liquid and pipette up and down to mix.
18. Vortex. Incubate all tubes on ice for 30 minutes.
19. First Wash: Add 500µL of 1X PBS in each tube and resuspend the pellet by pipetting up and down several times to mix. Use a new pipette for each tube. Vortex. Return tube back to ice.
20. Centrifuge samples for 5 minutes as in Step 7.
21. Remove the supernatant from each tube using a new plastic pipette for each tube. Place liquid in waste beaker. Return tubes back to ice.
22. Second Wash: Add 500µL of 1X PBS in each tube and resuspend the pellet by pipetting up and down several times to mix. Use a new pipette for each tube. Vortex. Return tube back to ice.
23. Centrifuge samples for 5 minutes as in Step 7.
24. Remove the supernatant from each tube using a new plastic pipette for each tube. Place liquid in waste beaker. Return tubes back to ice.
25. Add 50µl of 1:50 SA-PE to sample tube. Vortex to mix. Incubate on ice for 30 minutes. Add 50µl of Staining Buffer (SB) to the non-stained tube.
*****Turn off the lights for the steps 20 – these involve fluorescent-labeled reagents and need to be completed in the dark.*****
26. Repeat First Wash: Add 500µL of 1X PBS in each tube and resuspend the pellet by pipetting up and down several times to mix. Use a new pipette for each tube. Vortex. Return tube back to ice.
27. Centrifuge samples for 5 minutes as in Step 7.
28. Remove the supernatant from each tube using a new plastic pipette for each tube. Place liquid in waste beaker. Return tubes back to ice.
29. Repeat Second Wash: Add 500µL of 1X PBS in each tube and resuspend the pellet by pipetting up and down several times to mix. Use a new pipette for each tube. Vortex. Return tube back to ice.
30. Centrifuge samples for 5 minutes as in Step 7.

31. Remove the supernatant from each tube using a new plastic pipette for each tube. Place liquid in waste beaker. Return tubes back to ice.
32. Add 50μL of 1% Formaldehyde PBS per tube. Pipette up and down to mix.
33. Cover all tubes with aluminum foil, label with initials and date, and store at 4^{o} C until FACS.
34. On the day of FACS, (ideally the next day or same day as staining), add 400μL of 1X PBS per tube, vortex, and transfer into individual flow tubes. Keep all tubes on ice and in the dark.
35. Analyzing samples with flow cytometer to determine whether student sample was WT mice or CD40 KO mice.

XII. Teacher Answer Keys

A.

Bozeman Science - Immune System (Teacher)
http://www.bozemanscience.com/immune-system

Paul Anderson starts his video with an analogy of the immune system being like a castle.

1. The castle wall is like our ___skin___ that provides us with a ___barrier_____. It also has a _low__ __pH__ that makes it hard for bacteria to live there.
2. Chemicals on the surface or our skin and bacteria can also provide protection against ___infection.
3. Occasionally, you get a cut and it is equivalent to when the wall is breached.
4. Our bodies respond by _inflammation.
5. Our soldiers are the __macrophages or big eaters. The invaders are called __antigens. Something to remember is that macrophages attack -any invader.
6. The specific immune response is more like the __spies. The antigens have specific __proteins on its surface.
7. We have __antibodies that recognize antigens.
8. Antibodies are Y-shaped. At the top are two arms with different shapes that can dock to the _antigen surface. This _marks the antigen so the macrophages can find them.

Specific Immunity

1. Lymphocytes are a type of __white blood cell. Two types - B and T lymphocytes.
2. **B lymphocytes** are made in the _bone marrow and are responsible for a humoral response found in the fluids of your body.
3. B lymphocytes produce _antibodies. A näive B cell senses the _shape of an antigen and produces antibodies that are specific to the antigen surface.
4. Then memory cells are produced.
5. **T lymphocytes** are responsible for cell- mediated response that targets infected cells.
6. T lymphocytes are made in the _thymus and produce killer T cells. These dock to infected cells and kill our own cells.

Flow chart

Begin with an antigen that is has been eaten by a macrophage that chops it up into pieces. Pieces of the antigen are presented on the surface by _MHC2 chemical and then a T-_helper cell (CD4) docks to the antigen and initiates both the _humoral and _cell-mediated responses to produce B cells, antibodies, activate macrophages, and killer T cells.
Patients with _HIV don't have a normal immune response because the virus infects the _helper T cells.

B.

Name _____

ELISA Tutorial

A common tool used by scientists and researchers to test for the presence of an antibody or antigen is the enzyme-linked immunosorbent assay (ELISA). In fact, ELISA is used in home pregnancy and HIV tests. To understand how the test works, go to the *ELISA activity* on the Biology Project website and answer the following questions.

1. There are four main components to an ELISA. List each one below.
   _____inactivated antigens to coat plate_____
   _____Patient serum containing antibodies_____
   _____Secondary antibody (immunoglobulin + enzyme)_____
   _____Substrate that changes color _____

   Start the "Positive ELISA animation". (It moves quickly so you will need to stop it at times to answer the questions)
2. For an HIV test, what is the first component added to the ELISA plate?
   _____antigen_____
3. What is added next? _____patient serum_____
4. The next step is not clearly shown but involves washing the plate with buffers. What is the scientist trying to wash away? _____the antibodies that are not bond to the antigen
5. What is the third component added? _____second antibody_____
6. After the second wash is complete, what components are left in the plate? How are they connected? The antigen, patient antibodies, and secondary antibody are bound together.

7. The last step is to add a substrate. What are we looking for if the substrate reacts with the secondary antibody? _____a color change_____

8. Watch the "Negative ELISA test" animation. Explain what happens that causes the negative result (no color) to appear.
In the negative ELISA test, the patient serum does not contain the antibodies that recognize the antigen so they will not attach and will be washed away during the wash steps. The secondary antibody and enzymes will also be washed away because they are not able to attach. Therefore, no color will appear when you add the substrate because there is no enzyme for it to react with to produce the color.

9. What is meant by a "false positive"? A positive result that is not due to having the correct antibodies present.

10. Finish the tutorial by looking at the results of the 3 patients tested. Continue to the short 4 question quiz at the end.

C.

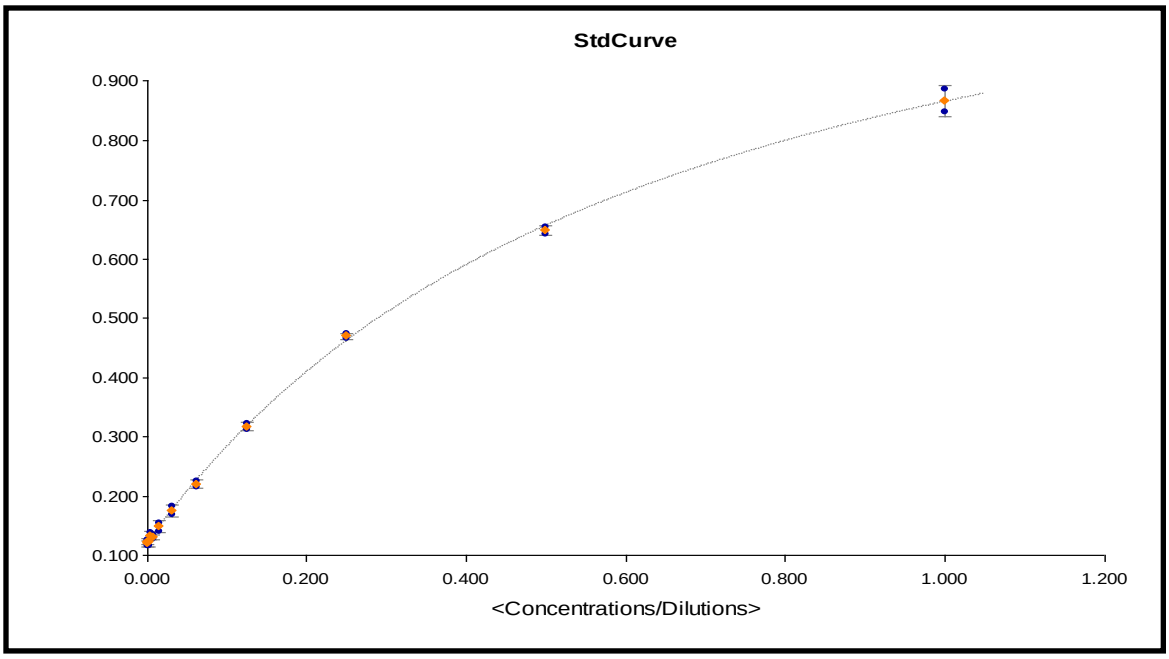
MAIDS Serum IgM ELISA

Analysis Questions:

1. Looking at your data, do you believe one of your samples comes from a mouse infected by the mouse type of AIDS (MAIDS)? Why?
In our experiment, sample A was from an infected mice. You can identify this sample by the higher concentration of the IgM in the mouse serum. You would expect the antibodies to be higher in an infected mouse than an uninfected mouse.

2. Summarize what happens in the well of the plate during an ELISA test. Include all components that would be found in a positive sample.
The plate is coated with a capture antibody that will bind to the target IgM molecule. Then the wells are blocked to keep the capture antibody from washing away. Mouse serum samples are loaded to each well. If IgM is present, it will bind to the capture antibody and not be washed away during the three was steps. Then a detection antibody is added that will bind to the other end of the IgM molecules that are present. A secondary antibody is added along with an enzyme that will react with the substrate that is added at the end. A yellow color will result in positive plates if all the molecules bind in the right order. No color will appear in wells with very low or no IgM present.

3. Sample Standard Curve



4. Find the concentration of IgM for the following 405 absorbance numbers for the following samples using the standard curve you graphed.

a. 0.64 Concentration 500ng/ml

b. 0.34 Concentration 160 ng/ml

c. 0.85 Concentration 900 ng/ml

D.

Name_____

Using Mice Models in Science

Part 1: "Knockout Mice"

Go to the NIH website and read "Knockout Mice" . Answer the following questions from each section.

What is a knockout mouse?

1. How is a knockout mouse made? _____ researchers use laboratory mice and inactivate or "knock out" a gene and replace it or disrupt it by inserting new DNA. _____
2. How does the mouse change when the genes are manipulated? _____ the mouse may have a new phenotype, behavior, or biochemical changes. _____

What are knockout mice used for?

3. Why are mice used to study human genes? Give two examples. _____ By knocking out genes in mice, researchers can gain information to better understand how diseases are contracted or to find possible cures. Examples will vary from the list on the website._____
4. How are mouse models named? Give an example of each.
_____Mouse models are named in two different ways. One is for the gene that is inactivated such as p53 knockout mice. Answers will vary. The second way they are named is by their physical characteristics or behaviors that occur such as "Frantic". Answers will vary. _____

What are the drawbacks of knockout mice?

5. Explain the two limitations that present themselves in knockout mice.
_____ 15% of the gene knockouts are lethal so don't grow to adulthood so sometimes this is a problem when the study is concerned with adult human diseases. The gene would also act differently in adults than in embryos. Knockout genes sometimes don't show an observable change in a mice even if it causes a change in humans.____

How are knockout mice made?

6. Why do researchers use embryonic stem cells to create the knockout mice?

_____ ES cells can differentiate into any cell in the body so you can study it in adults too. Researchers can keep cell lines to created knockout mice in the future._____

7. What is meant by "in vitro"?

_____ made in the lab_____

8. The first strategy discussed is gene targeting or homologous recombination. Go to Nobelprize.org and read through the procedure. Summarize this strategy in your own words.

_____ Answers will vary. Basic procedure: Harvest mouse ES cell, create a target vector and introduce target gene to ES cell, homologous recombination allows vector to find gene, select cells with target gene and culture cells. _____

9. How is gene trapping different from gene targeting?

_____ The DNA is inserted randomly and shuts down the cell's RNA "splicing machinery" so it doesn't work properly and the gene isn't knocked out. _____

10. What are homozygous knockouts?

_____ Mice that have been crossbred so that both copies of the gene are knocked out.

Which production method is best?

11. Looking at the advantages and disadvantages of each strategy, which do you believe is better and why?

_____ Answers will vary_____

Part 2: "Transgenic Mice"

Go to the Learn Genetics website and read the information on transgenic mice. Then answer the following questions.

1. Who won the Nobel Prize in 2007? What field of study was the research in? _____Mario R. Capecchi, Physiology or Medicine _____
2. What question did he raise regarding the future of medicine? _____"If we can replace a perfectly good gene with a mutated one, can we also go the other way, replacing problem genes with those that work?"_____
3. What do homeotic genes code for? _____body patterns _____

4. Create a flow chart below showing the steps to making a knockout mouse.

Isolate ES cells from mouse with normal gene →Add a mutated gene with a drug-resistance marker →genes swap places by homologous recombination to create the knockout →add drug that would kill cells that do not contain the drug-resistance marker →transplant the ES cells to mice →grow mice and breed to create knockout mice.

E.

Name_____

Flow Cytometry
Online Tutorial Worksheet

As you watch the tutorial *Molecular Probes Tutorial Series – Introduction to Flow Cytometry,* answer the following questions.

1. What does the flow cytometer use to count cells and analyze them?

Light/laser beam

2. What can the flow cytometer tell a scientist about a cell?

Size, complexity, phenotype, and health

3. What is important about the way the cells are transferred into the interrogation point?

Particles and cells must go through one at a time

4. How big are the cells that the flow can detect?

1-15 microns in diameter

5. When cells go through the laser and present a forward scatter pattern, it can determine the __size_ of the cell.

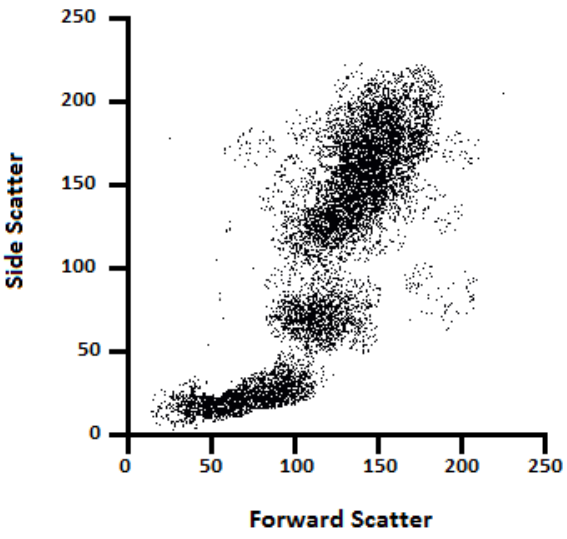
6. The scattered light received by the detector will send data to a computer. This can show the size of cells. Explain from this histogram if the majority of cells in the sample are large or small. How do you know?

The majority of cells are larger due to the peak in the right hand corner of the histogram.

7. When light is scattered at larger angles and off to the side, it is called a side scatter. What causes this side scatter?

Granularity and structural complexity inside the cell

8. Plotting a two dimensional scatter plot can tell you more than a one dimensional histogram. Label the 2D scatter plot below with the types of cells you would expect in a blood sample.



http://aws.labome.com/figure/te-125-10.png

neutrophils

monocytes

lymphocytes

9. Fluorescence occurs when fluorophore are ____excited___ and then return to their _ground state_____. The energy in the emitted light is dependent on the energy level that it is excited and it relates directly to ____wavelength_____ and _____color____.

10. Studying specific cells can be done using a fluorophore-labeled ___antibody___ that will bond to specific cells and reading data collected by the flow cytometer. Fluorescence data is collected by the amount of light detected.

11. Why is a threshold important?
There are more than just the cells of interest being passed through the laser and are not needed for data points and are not cells of interest to the researcher.

Analyzing Flow Cytometry Data
Watch the tutorial *Molecular Probes Tutorial Series – Analyzing Flow Cytometry Data*.

1. As cells pass through the laser, the light pulse is converted into a ____voltage_____ pulse that is then converted into a __numerical_____ value.
2. There are 3 ways to measure the pulse. What are they and what can they tell you?
    1) Height - quantify size of voltage pulse
    2) Area - quantify size of voltage pulse
    3) Width - measure DNA analysis

3. What two types of graphs are used? Histograms or dot plots

4. In our upcoming field trip to the UNE lab, you will be detecting whether you have cells from a wild-type mouse or one that is a CD4 Knockout mouse. Based on the tutorial, would you use a linear or log scaling? Why?

You would use the log scaling so that the population showing CD4 cells would be spread out and the negative population would not be compressed along the axis.

5. What does gating allow you to do?

Restrict analysis to a specific cell population

6. In the plot below, which cell type population are we gating?
neutrophils

7. How is compensation calculated for the two color samples?
Subtract the % of one color that is overlapping into the other color
R-PE - % Alexa Fluor 488

STOP VIDEO AT 9:50.