The Lymphatic System: Structure and Function with a Focus on T Cell Activation

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I. Abstract

This unit is designed to fit into an anatomy/physiology course or an advanced high school biology curriculum (i.e. AP Biology). It begins by introducing the structure and function of the lymphatic system, but mostly focuses on the function of the immune system, with an emphasis on T cell activation. It culminates with a lab utilizing an ELISA to determine the extent of T cell activation in a sample and apply this to a real-world research concept.

II. Science Background

a. Instructor
    i. understand the basic workings of the immune system
    ii. describe in more detail how T cells are activated…this is necessary for the completion of the main laboratory exercises included in this unit
    iii. understand how an ELISA works
b. Student
    i. no knowledge of the immune system is necessary going into this unit
    ii. should have a basic understanding of cells, taught during a general biology unit

III. Student Outcomes

a. concepts covered
    i. parts of the immune system
    ii. adaptive vs. innate immunity
    iii. activation of immune cells
    iv. vaccination
    v. epidemiology and epidemics
    vi. ELISA laboratory techniques
b. NGSS or other standards
    i. NGSS:
        1. HS-LS1-1. 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.
        2. HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
c. placement within high school curriculum
    i. This unit will fit well into any anatomy and physiology course curriculum.
    ii. This unit could also apply to the AP curriculum. All or some of the unit could be used in the cell signaling and cellular communication section.
d. what can students do and what technical skills will they learn
    i. Students will learn the structures and cells of the lymphatic/immune system.
    ii. Students will understand the difference between the innate and adaptive immune responses and how they are connected to each other.
    iii. Students will explain how the different types of immune cells are activated.
    iv. Students will connect the activation of T cells to a real-world research scenario.
    v. Students will learn how to work with micropipettes.
    vi. Students will perform an ELISA and be able to accurately communicate what the results mean when applying it to the real-world research scenario in the form of a formal laboratory report.
e. relevance to other science concepts and students' lives

i. This unit relates to other basic cell biology concepts; cell structures, cell signaling and cellular communication.
ii. This unit includes a discussion of epidemics and vaccines which are relevant with current events happening throughout the world (emergence of new diseases (e.g. coronavirus), decline in vaccinations among children, etc.)

IV. Learning Objectives

a. Students will be able to:
   i. identify the different structures of the lymphatic system and how they are connected to the cardiovascular system
   ii. describe the functions of each of the different cells of the immune system
   iii. describe the innate immune response and its connection to the adaptive immune response
   iv. explain how T cells are activated and their role in the adaptive immune response
   v. describe the structure and functions of antibodies
   vi. explain how vaccination leads to immunization
   vii. define the different types of vaccines
   viii. explain the difference between and epidemic and pandemic
   ix. trace a disease through a population and determine patient zero
   x. explain how an ELISA works to detect an antigen
   xi. perform an ELISA and determine which samples contain the antigen
   xii. apply the results of an ELISA to a real-world research scenario, where they will determine if a certain drug is effective at suppressing the activation of T cells

V. Time Requirements

a. This unit was designed to be taught in 45 minute class periods.
b. The unit includes 10 lessons, covering about 11-12 days of class. This can be modified if more time is needed for certain lessons.

Day	Concepts Covered	Activities/Homework
1	• Functions of the lymphatic system • Lymphatic structures (vessels, path of lymph, thymus, spleen, other lymphatic tissue)	• Chapter Notes • Lymphatic System Worksheet (labeling and vocabulary practice)
2	• Briefly describe innate and adaptive immunity • Describe the cells of the immune system	• Chapter Notes • Immune Cell Memory Game
3	• Innate immunity	• Mile-a-Minute Game • Quiz – Lymphatic Structures and Cells of Immune System • Chapter Notes
4	• Adaptive immunity ○ Cellular response and T cell activation	• Chapter Notes • Adaptive Immune Response Comic Book Project

5	• Adaptive immunity ○ Humoral response and B cell activation	• Chapter Notes • Adaptive Immune Response Comic Book Project (continue from previous day)
6	• Vaccines	• Chapter Notes • "Vaccines: Calling the Shots" Video • Influenza Case Study (homework)
7	• Epidemiology and Epidemics	• Epidemiology Notes • Plague Lab
8	• ELISA concepts and procedure	• Quiz – Innate and Adaptive Immunity • HIV and ELISA notes • Pre-Lab Questions
9	• ELISA and T cell activation/proliferation	• T Cell Activation/ELISA Lab
10	• ELISA and T cell activation/proliferation	• Complete ELISA Lab • Begin Written Lab Data and Discussion
11	• ELISA and T cell activation/proliferation	• Complete Written Lab Data and Discussion

VI. Materials and Equipment

a. Plague Lab
   i. This lab activity requires the following materials:
      1. large Dixie cups (enough for every student in the class)
      2. flour
      3. baking soda
      4. vinegar

b. ELISA Simulation Kit
   i. Purchased from Carolina Biological ($128.00) https://www.carolina.com/biotechnology-elisa-kits/elisa-simulation-kit/FAM_211248.pr
   ii. Kit includes:
      1. positive and negative controls
      2. simulated patient samples
      3. simulated antigen

4. simulated secondary antibody
5. simulated chromagen
6. plastic pipettes
7. microtiter plates
8. teacher manual (includes student guides)

iii. This kit is designed for 32 students working in pairs.

iv. Parts of this kit can be reused (microtiter plates) for the next year. There are refill kits available from Carolina Biological for $64.00 which includes the simulated samples and more plastic pipettes.

c. BioRad ELISA Immuno Explorer Kit

i. Purchased from BioRad ($138.00) https://www.bio-rad.com/en-us/product/elisa-immuno-explorer-kit?ID=1e3f3100-99f6-49b3-b9a0-2c8aad9d9285

ii. Kit includes:

1. antigen (chicken γ-globulin)
2. primary antibody (rabbit anti-chicken polyclonal antibody)
3. secondary antibody (goat anti-rabbit antibody, conjugated to horseradish peroxidase HRP)
4. HRP enzyme substrate, 25 ml
5. 10x PBS, 100 ml
6. 10% Tween 20, 5 ml
7. disposable plastic transfer pipettes (80)
8. microplates with 12-well strips (8 rows of 12 wells, 3 count)
9. yellow micro test tubes, 2 ml (60)
10. colored micro test tubes, 2 ml (75)
11. teacher guide (includes student manual)

iii. Other required equipment

1. adjustable micropipettes (20-200 µl) or fixed volume micropipettes (50 µl)

d. Precautions and Safety

i. Lab safety equipment required

1. gloves
2. goggles

ii. Storage

1. ELISA Simulation Kit
    a. The samples for this kit are perishable and need to be stored in the freezer or refrigerator.
2. BioRad ELISA Immuno Explorer
    a. Some of the materials for this kit must be stored in the refrigerator.
    b. Once set up, the solutions need to be kept on ice or in the refrigerator if prepared more than 4 hours in advance.

VII. Advance Preparation

a. Preparation

i. Refer to kit instructions for information on preparing solutions for the laboratory exercises.

1. For the ELISA Simulation kit, use the HIV section of the student instructions.

2. For the Immuno Explorer kit, follow Protocol II for the antigen detection ELISA. You will need to decide whether you want the results of the lab exercise to show the experimental drug is effective or not. If you would like it to be effective, you will not add the antigen to samples 1 and 2 (the ones which received the drug).

ii. For the plague lab, you will be preparing enough cups for each student in your class. This can be done the day before, but the cups will need to be mixed a little bit right before class starts. Label each cup with a shape or a letter (depending on how many students are in the class). Fill each cup half full of water and some baking soda to one of the cups. This will be the individual who will start with the infection. Write down which cup is the infected cup so you will know if the students correctly trace the infection through the classroom. Then add some flour to all of the cups and mix well. Be careful not to cross-contaminate between the infected cup and the other cups.

b. Approximate prep time
   i. Plague Lab – approximately 30 minutes to prepare the cups for students
   ii. Carolina Kit – approximately 1 hour to aliquot the materials
   iii. BioRad Immuno Explorer – approximately 1-2 hours to aliquot the materials

VIII. Student Prior Knowledge and Skills

a. expected prior content knowledge
   i. Students are expected to have a basic understanding of biology coming into this unit. Knowledge of cells and cell parts is necessary.
b. expected prior technical skills
   i. Students do not need any technical skills to complete this unit. Depending on equipment available, knowing how to use a micropipette would be a useful skill. The laboratory exercises can use either the disposable pipettes or micropipettes.
c. possible preconceptions
   i. Prior to this unit, students have not been exposed to much of this content. Some may have discussed cell signaling if they have taken an AP Biology course, but most of the content we cover is new.
   ii. Some students may have background knowledge on vaccines, at least from what they learn outside of school. When discussing the topic of required vaccinations you may need to be careful when discussing mandatory vaccinations. Emphasizing the fact that herd immunity is the only way to protect those who are unable to vaccinate is helpful.

IX. Daily Unit Plans

a. Day 1 – Introduction to the lymphatic system
   i. Objectives:
      1. list the structures of the lymphatic system and explain their functions
      2. describe the functions of the lymphatic system
   ii. Activities:
      1. chapter PowerPoint notes (slides 1-12)
      2. lymphatic structures review worksheet (Appendix A)
b. Day 2 – Cells of the Immune System/Adaptive vs. Innate Immunity
   i. Objectives:
      1. explain the differences between adaptive and innate immunity
      2. describe and identify the different cells of the immune system

ii. Activities:
    1. chapter PowerPoint (slides 13-19)
    2. immune cell memory game (Appendix B)

c. Day 3 – Innate Immunity
    i. Objectives:
        1. describe the different types of innate immune response (first line and second line of defense)
    ii. Activities:
        1. Mile-a-Minute review game (Appendix C)
        2. chapter PowerPoint (slides 20-26)
        3. exit slip (Appendix D)

d. Day 4 and 5 – Adaptive and Innate Immunity
    i. Objectives:
        1. describe the humoral and cell-mediated immune response
        2. explain the process of B cell and T cell activation
        3. explain the differences between the two different responses and how that relates to the overall response to a pathogen
    ii. Activities:
        1. chapter PowerPoint (slides 27-44)
        2. adaptive immune response comic strip project (Appendix E)

e. Day 6 – Vaccines
    i. Objectives:
        1. describe the various types of vaccines
        2. understand how vaccines can protect against infection by a pathogen
        3. explain why herd immunity is important in protecting the entire community from infection
    ii. Activities:
        1. chapter PowerPoint (slides 45-49)
        2. "Vaccines: Calling the Shots" video worksheet (Appendix F)
            a. Video can be accessed from PBS https://www.pbs.org/wgbh/nova/video/vaccinescalling-the-shots/
        3. A Case Study Involving Influenza and the Influenza Vaccine (assigned as homework)
            a. Can be accessed from the National Center for Case Study Teaching in Science https://sciencecases.lib.buffalo.edu/collection/detail.html/?case_id=326&id=326

f. Day 7 – Epidemiology and Epidemics
    i. Objectives:
        1. understand the job of an epidemiologist
        2. explain the difference between and epidemic and pandemic and give examples of each
        3. trace a disease through a population and determine patient zero
    ii. Activities:
        1. Epidemiology PowerPoint (Appendix G)
        2. Plague Lab (Appendix H)

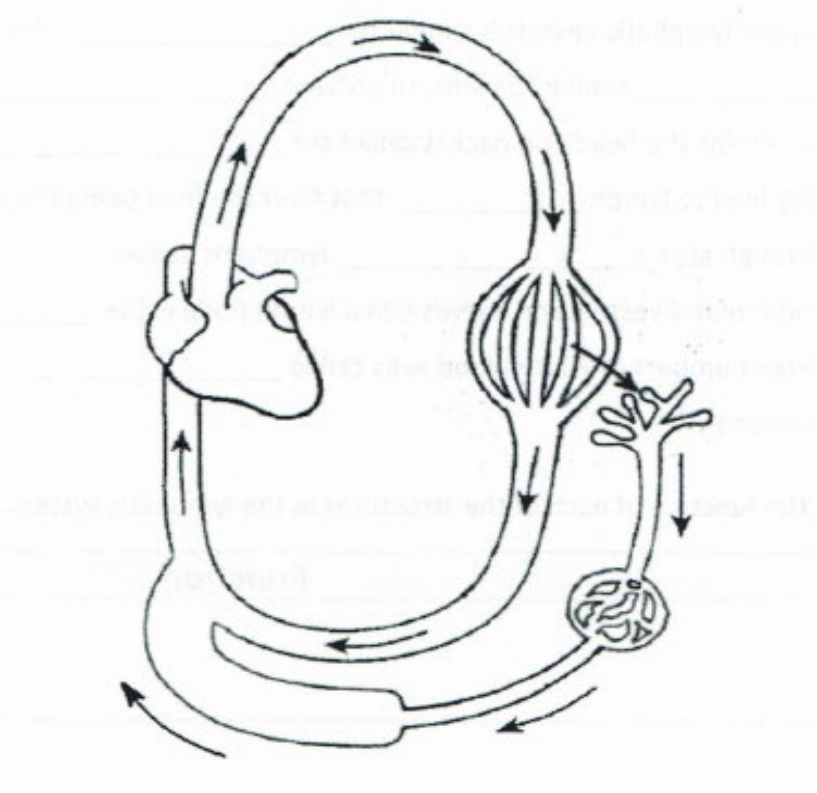
g. Day 8/9 – HIV/AIDS and ELISA Concepts and Procedure

i. Objectives:
    1. understand how an ELISA is used to determine the presence of an antigen
    2. describe the steps of an ELISA in terms of determining if a patient has been exposed to HIV
    3. complete an ELISA for several patients and determine if they have contracted HIV
    4. explain the purpose of the positive and negative controls in an ELISA
ii. Activities:
    1. HIV/AIDS and ELISA PowerPoint (Appendix I)
    2. ELISA Simulation Kit from Carolina Biological

h. Day 10 and 11 – T Cell Activation Lab
    i. Objectives:
        1. perform an ELISA to determine the extent of T cell activation in various samples
        2. explain how the results from the ELISA relate to the real-world research scenario (how effective is a drug designed to suppress T cell activation)
        3. communicate these results in the form of a formal lab report
    ii. Activities:
        1. Determining Activation of T Cells with an ELISA laboratory exercise (Appendix J)
        2. formal lab report outline (Appendix K)

Appendix A
The Lymphatic System Worksheet

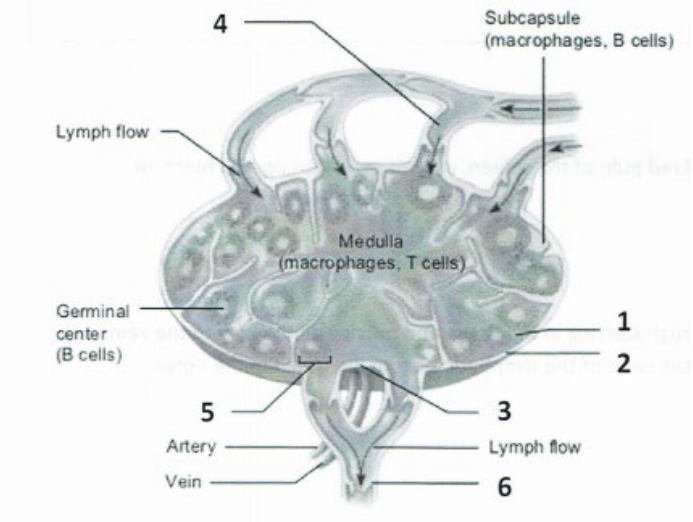
Using the list to the right, label the parts of the lymphatic and circulatory system on the diagram below.



- Artery
- Blood Capillary
- Vein
- Heart
- Lymphatic Capillary
- Lymphatic Duct
- Lymph Node
- Lymphatic Vessel

On the diagram above, color the following structures:

1. Color the arteries red
2. Color the veins blue
3. Color half the capillary bed red and the other half blue
4. Color the lymphatic capillaries, lymph node, lymphatic vessels, and lymph duct green



Label the diagram of lymph node by writing the correct numbers in the spaces provided:

_____ Afferent lymphatic vessel

_____ Efferent lymphatic vessel

_____ Capsule

_____ Hilum

_____ Nodule

_____ Sinus

Fill in the Blank

1. Lymphatic pathways begin as _____ that merge to form lymphatic vessels.
2. The wall of a lymphatic capillary consists of a single layer of _____ cells.
3. Once tissue fluid is inside a lymphatic capillary the fluid is called _____.
4. The structure of the walls of lymphatic vessels is similar to _____, but thinner.
5. Lymphatic also have _____ , similar to veins, to prevent _____.
6. The lymphatic trunk that drains the head and neck is called the _____ trunk.
7. Lymphatic vessels usually lead to lymph _____ that filter the fluid being transported.
8. Lymph enters a node through a(n) _____ lymphatic vessel.
9. The indented region where blood vessels and nerves join a lymph node is the _____.
10. Lymph nodes contain large numbers of white blood cells called _____ and macrophages that fight invading microorganisms.

In the table below, summarize the function of each of the structures in the lymphatic system.

Structure	Function
Red Bone Marrow	
Thymus	
Spleen	
Lymph Nodes	

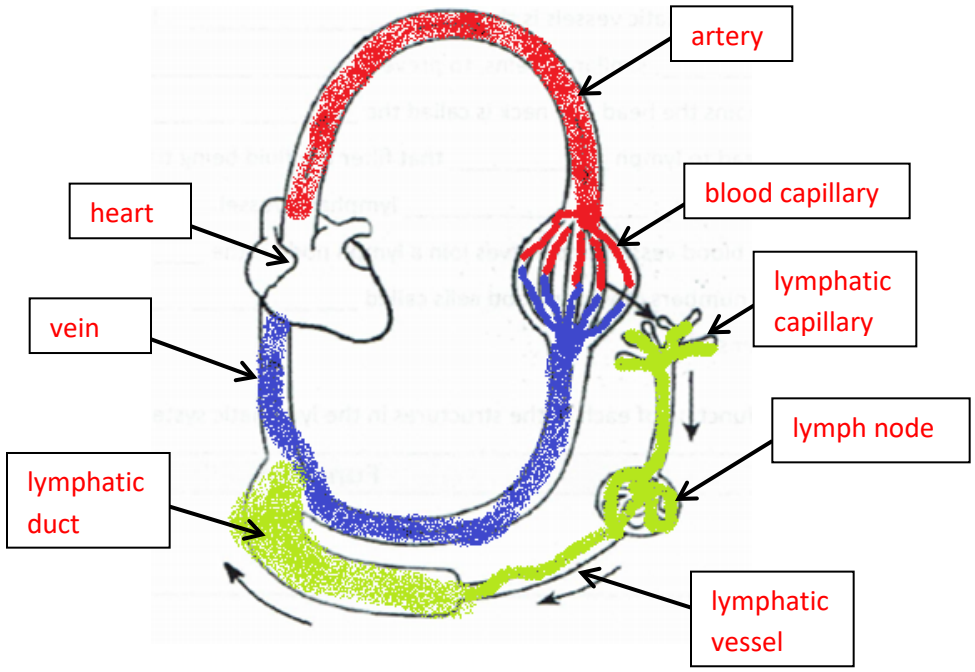
Short Answer

11. Explain the difference between the white and red pulp of the spleen, in both cell make up and function.

12. List the structures tissue fluid would pass through starting in the tissue and moving all the way to the veins (cardiovascular system). Include all the different parts of the lymphatic system discussed in the notes

The Lymphatic System Worksheet (KEY)

Using the list to the right, label the parts of the lymphatic and circulatory system on the diagram below.



- Artery
- Blood Capillary
- Vein
- Heart
- Lymphatic Capillary
- Lymphatic Duct
- Lymph Node
- Lymphatic Vessel

On the diagram above, color the following structures:

1. Color the arteries red
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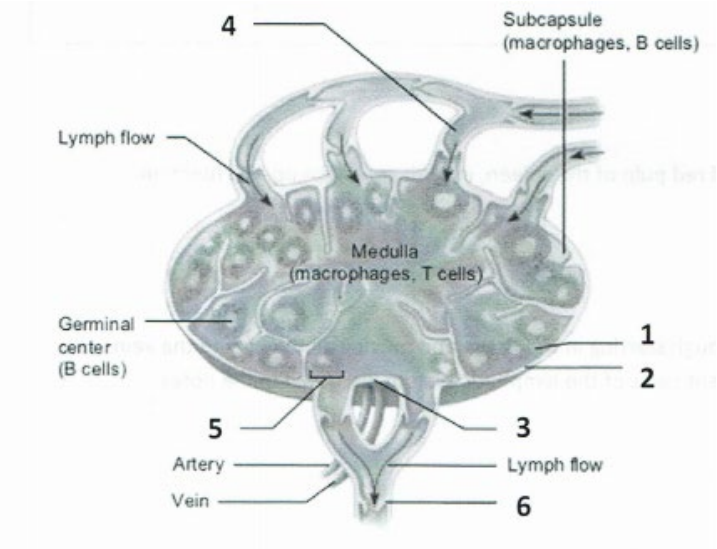
4. Color the lymphatic capillaries, lymph node, lymphatic vessels, and lymph duct green



Label the diagram of lymph node by writing the correct numbers in the spaces provided:

__4___ Afferent lymphatic vessel

__6___ Efferent lymphatic vessel

__2___ Capsule

__3___ Hilum

__5___ Nodule

__1___ Sinus

Fill in the Blank

1. Lymphatic pathways begin as capillaries that merge to form lymphatic vessels.
2. The wall of a lymphatic capillary consists of a single layer of epithelial cells.
3. Once tissue fluid is inside a lymphatic capillary the fluid is called lymph.
4. The structure of the walls of lymphatic vessels is similar to veins, but thinner.
5. Lymphatic vessels also have valves, similar to veins, to prevent backflow.
6. The lymphatic trunk that drains the head and neck is called the jugular trunk.
7. Lymphatic vessels usually lead to lymph nodes that filter the fluid being transported.
8. Lymph enters a node through a(n) afferent lymphatic vessel.
9. The indented region where blood vessels and nerves join a lymph node is the hilum.
10. Lymph nodes contain large numbers of white blood cells called lymphocytes and macrophages that fight invading microorganisms.

In the table below, summarize the function of each of the structures in the lymphatic system.

Structure	Function
Red Bone Marrow	blood cell and lymphocyte production
Thymus	site of T cell maturation
Spleen	filters the blood, contains red and white pulp
Lymph Nodes	filters pathogens and other foreign materials from the lymph

Short Answer

11. Explain the difference between the white and red pulp of the spleen, in both cell make up and function.

red pulp: site of red blood cell production, makes up the majority of the spleen
white pulp: houses lymphocytes, site of antigen presentation

12. List the structures tissue fluid would pass through starting in the tissue and moving all the way to the veins (cardiovascular system). Include all the different parts of the lymphatic system discussed in the notes.
    lymphatic capillaries, lymphatic vessels, lymph nodes, lymphatic duct, vein, heart

Appendix B

macrophage	engulf/kill invading microorganisms; help induce inflammation; can activate other immune cells
mast cells	play a role in allergic responses; can respond to parasitic worms; can induce inflammation
neutrophils	phagocytic granulocytes; play a role during bacterial infection

eosinophils	defend against helminth worms and bacteria; are associated with allergic reactions
basophils	defend against parasites; associated with allergic reactions
dendritic cells	bridge between innate and adaptive immunity; mostly involved with antigen presentation; can take up and degrade pathogens

natural killer cells	not antigen-specific; can destroy some abnormal cells such as tumor cells and some virus-infected cells
B cells	divide to form plasma cells when activated; produce and secrete antibodies
helper T cells	initiate responses from other cells in the immune system

cytotoxic T cells	kill cells infected with a virus or other pathogen
regulatory T cells	suppress immune activity and help control immune response

Appendix C

Mile-a-Minute Vocabulary Review Game

This vocabulary game is easy to do and only requires you to post a list of vocabulary words on the board or screen. Students will be working in pairs to go through and review each of the vocabulary words. It also holds students accountable for their knowledge since the winning pair will have to come up to the front and define the words to show the class they actually know them.

Procedure:

1. Post a list of words on the board or screen. The vocabulary words to review at this point in the unit are listed below. These will show up on their quiz.
    a. lymph node
    b. lymph
    c. thymus
    d. spleen
    e. helper T cells
    f. cytotoxic T cells
    g. B cells
    h. macrophage
    i. mast cells
    j. neutrophils
    k. eosinophils
    l. basophils
    m. dendritic cells
    n. natural killer cells
    o. regulatory T cells
2. Split students into pairs; one student will be the guesser and the other will be the clue-giver. Students will start the activity standing and sit down when they have completed the list.
3. The clue-giver will choose a word from the list and give the guesser clues to help them determine which word they chose. You could allow students to use their notes, but if it is used for review allowing notes may not be as effective.
    a. Students can't use clues like "it is the first word on the list".
    b. Students can't use clues such as "rhymes with *monosynthesis*".
4. Once the first guesser correctly identifies the words, students switch roles and start the game again.
5. As soon as both students in the pair have gone through the words, they sit down so you know they are done. Write down the order of the groups as they finish.
6. Once everyone is done, the team that finished first must define the words from the list. If they do not correctly define the words, the next team has a chance to define them. The team that can correctly define all the words is the winner. This step allows you to hold groups accountable for knowing the words.

Appendix D

Exit Slip – Innate Immune Response

1. Why is the innate response also called non-specific?

2. What is considered the first line of defense? Explain why.

3. Explain the relationship between the innate and adaptive immune responses.

Match the following words with their definitions:

4. _____ localized pain, heat, redness, and swelling
5. _____ large cell that can engulf and destroy pathogens
6. _____ proteins that attract cells to an area
7. _____ a cascade of proteins that promotes inflammation and destruction of pathogens
8. _____ proteins which can affect the behavior of nearby cells

a. macrophage
b. cytokines
c. chemokines
d. inflammation
e. complement

Appendix E

Adaptive Immune Response Comic Strip

Objective: Design a cartoon that illustrates the adaptive immune response and the functioning of the immune system.

Requirements:

- Introduce an invader: bacteria or virus
- Represent the invaded cell or body
- Describe the location and function of B cells.
- Describe the location and function of the different types of T cells (listed below).
    - helper T cells
    - cytotoxic T cells
    - memory cells
- Represent both antigens and antibodies.
- Describe the role of the macrophages.
- Your story should illustrate how invaders are recognized and immune memory is maintained.
- It should include *at least* 8 plates or scenes.
- Be Creative, Colourful and Imaginative!

Grading Rubric

Requirement	Points Earned
Adaptive immune response explained correctly ___/10	
Players and their roles correctly identified ___/5	
Story line is clear, correct, and easy to follow ___/5	
Effort was put into the project (drawings are clear and colorful) ___/5	
Total Points: _____ /25	

Appendix F

Name: _____ Period: _____ Date: _____

Vaccines: Calling the Shots Video Worksheet

1. Before watching the video, for each of the statements below, indicate whether you agree (A) or disagree (D). After watching the video, do the same thing in the next column and see if any of your opinions have changed.

Before (agree or disagree)	After (agree or disagree)	Statement
		Parents should be required to vaccinate their children when starting them in daycare (currently not required in Minnesota).
		People who work with children of any age should be required to have and be up to date on all required vaccinations.
		The risk of side effects is high enough to allow people to make the decision to not vaccinate their children.
		Parents who choose to space out their child's vaccinations should still be allowed to enroll them in school/daycare, even if they haven't received all of the required vaccines at that point in time.
		I would vaccinate my children.

2. What is pertussis? What are some of the symptoms of this illness? Why is this illness so dangerous to infants and small children?

3. Why do outbreaks of preventable diseases occur in a country where these diseases have previously been eradicated?

4. List 2 reasons why parents choose to delay vaccinations or not vaccinate their children at all?

5. Five-hundred years ago, how many children died before the age of 5?
6. Briefly summarize the history of vaccinations.

7. Explain what herd immunity is and why this is important for certain individuals in a population. What percent of the population needs to be vaccinated to maintain herd immunity?

8. What did Luke's medical team find to be the cause of his seizures? Explain what triggers these seizures.

9. What are some common side effects of vaccinations?

10. In 1994, what was the only cause of Polio cases in the U.S.?

11. What do medical experts believe causes autism?

12. How many people will "catch" human papilloma virus (HPV)?

13. What cancers are caused by HPV?

14. What is the chance of having a severe allergic reaction to a vaccine?

15. Go back to the table in question #1 and fill in the after column. Did you opinions change based on the information in the video? Do you feel like you need more information to make a decision?

Appendix G (Epidemiology PowerPoint Slides)

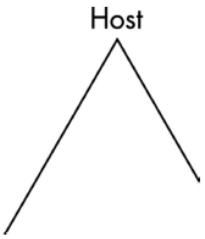
Epidemiology and Epidemics

What is epidemiology?

- study of the distribution and determinants of health problems populations
  - applying that information to control health problems
  - who diseases affect, what factors are involved, and how can it be contained

Epidemiologic Triangle

- Agent (What)
  - microbe that causes the disease
- Host (Who)
  - organism harboring the disease
- Environment (Where)
  - external factors which allow or cause disease transmission



Epidemics and Pandemics

- epidemic
  - more cases of a particular disease than expected in a given area/population
  - example:
    - Polio 1950s – 58,000+ cases and 3,145 deaths
- pandemic
  - a widespread epidemic across a large region
  - examples:
    - Influenza 1918 – infected about 1/3 of the world population, about 100 million deaths worldwide
    - HIV/AIDS
    - Black Death 1347 – killed about 1/3 of European population

Ebola

- hemorrhagic fever
- symptoms include:
  - fever
  - severe headache
  - muscle pain/weakness
  - fatigue
  - vomiting and stomach pain
  - unexplained hemorrhage
- incubation period is between 2-21 days (avg. 8-10)
- recovery depends on care
- people who recover develop antibodies that can last for 10 years

Zika

- Spreads mostly through mosquitos
- Symptoms include:
  - Fever
  - Rash
  - Joint pain
  - Red eyes
  - Headache
  - Muscle pain
- Many people don't get sick enough to go to the doctor…some are asymptomatic
- Dangerous if infected during pregnancy
  - Linked to microcephaly and other severe brain defects in infants

Nipah

- Transmitted through direct contact with pigs, bats, or infected people
- Symptoms include:
  - Fever
  - Headache
  - Drowsiness
  - Disorientation and confusion
- Can progress to a coma within 24-48 hours
- Long term effects include persistent convulsions and personality changes
- 1999 outbreak nearly 300 infected with over 100 deaths
- Annual outbreaks in Bangladesh are common

Appendix H

Plague Lab

Introduction:

There is this disease this is spreading through your population at an alarming rate. The disease is transmitted by casual contact or by the exchange of "body fluids." In your immediate population there are two people who are already infected. Today we will demonstrate how quickly a disease can spread.

Hypothesis:

If two people in the class are infected at the start of the activity, how many people do you predict will be infected at the end of the activity? _____

Procedure:

1. You will receive a container with liquid, which will represent your "body fluid" by pouring the contents of your container into the other container of the other person. Pour half of the liquid back into your container. Now record the name of the contact person on your data sheet. Repeat this procedure with two more people and record the names of your casual contacts.
2. After all of the class population have made their four contacts, all class members will be tested to see who is infected. Remember, only two people are infected at the start.
3. Fill in the data table and construct a bar graph of your results.

Data Sheet

Fill in the results and observations:

Your Name	Contact 1	Contact 2	Contact 3	Contact 4

Class Results:

Student	Positive/negative	Student	Positive/negative	Student	Positive/negative
1		11		21	
2		12		22	
3		13		23	
4		14		24	
5		15		25	
6		16		26	
7		17		27	
8		18		28	
9		19		29	
10		20		30	

Summary of class results:

In the beginning	At the end
# students in class =	
# students infected =	# students infected =
% infected =	% infected =
% not infected=	% not infected =

Analysis and Conclusion

1. Were you infected at the end?

2. What percentage of the class was (show your work!):

    a. Originally infected?

    b. Finally infected?

3. Why were some people not infected by the disease?

4. In your own personal contact circle, at the end, how many people were infected out of the four?

5. Exchange information and see if you can trace the original two people who were infected.

Predicted infected person _____
Actual infected person _____

6. Why is it important to find the source of a disease?

7. If you were to come into contact with more people, how might this affect your ability to determine patient zero?

8. If a fatal virus spread through a community and there was a limited amount of medication for treatment, how would you decide who would receive treatment? Explain your reasoning.

Appendix I (HIV/AIDS and ELISA PowerPoint)

HIV and ELISA Testing

HIV – Human Immunodeficiency Virus

- retrovirus
  - uses reverse transcription
    - RNA to make viral DNA
- attacks the Helper T Cells (also called CD4)
- host cell killed as viruses release

HIV Affects Helper T Cells

- helper T cells are also known as CD4 cells
- normal counts range from 500 to 1500 cells per cubic millimeter of blood (cells/mm^3)
- initially in HIV infection there is a sharp drop in the CD4 count
  - count levels off to around 500-600 cells/mm^3

Transmission

- sexual transmission
- sharing contaminated needles
- blood products
- from mother to baby from birth or nursing

Progression/Stages of HIV

- acute infection stage
  - 2-4 weeks after infection
  - many experience flu-like symptoms
  - body's natural immune response to infection
- clinical latency stage
  - experience no HIV-related symptoms
  - HIV continues to reproduce at very low levels

- AIDS
  - autoimmune deficiency syndrome
  - HIV positive with a CD4 cell count below 200 cells/mm^3
  - HIV has damaged the immune system so people are more vulnerable to opportunistic infections
  - without treatment people who progress to AIDS survive about 3 years
  - with treatment some people will never progress to AIDS and will have a normal life expectancy

Factors Affecting Disease Progression

- factors that may shorten time between HIV and AIDS
  - older age
  - HIV subtype (different strains)
  - co-infections with other viruses
  - poor nutrition
  - severe stress

- factors that may lengthen the time between HIV and AIDS
  - antiretroviral therapy
    - HIV "Cocktail"
      - nucleoside reverse transcriptase inhibitors (NRTI)
        - » competes with the enzyme to stop reverse transcription
      - non-nucleoside reverse transcriptase inhibitors (NNRTI)
        - » inhibits the enzyme to stop reverse transcription
      - protease inhibitors
        - » blocks the cleaving of proteins required for the virus to mature
      - entry/fusion inhibitors
      - integrase inhibitors
        - » blocks the integration of viral DNA into host cell genome

Statistics

- In 1995, the number 1 cause of death for ages 25 – 44 in U.S
- As of December 31, 2011, a cumulative total of 9,785 persons have been diagnosed and reported with HIV infection in Minnesota.
  - 3,788 persons have been diagnosed with HIV infection (non-AIDS)
  - 5,997 have progressed to AIDS
- Of these 9,785 persons, 3,347 are known to be deceased

Data Taken from MN Department of Health

ELISA Testing

- Enzyme-Linked Immunosorbent Assay
- based on immune system antibodies
- can detect antibodies in someone's blood serum or the presence of an antigen/protein in a sample
- several applications:
  - diagnostic tool in medicine (diseases, pregnancy)
  - plant pathology
  - quality control in industry
  - forensics

How does an ELISA work?

- uses an antibody that is linked to an enzyme
  - enzyme will then react with its substrate and create a color change
  - color change = positive result
- if used to detect the antigen itself…
  - one antibody linked with an enzyme is used

- if used to detect presence of antibodies…
  - a secondary antibody is used (linked to an enzyme)
  - primary antibody is in the serum sample
  - secondary antibody binds to the primary antibody

Using ELISA to detect HIV infection…

- most common HIV test
- detects the presence of HIV antibodies
- after 4-8 weeks of exposure, the body will have produced detectable levels of antibodies for the HIV protein antigens

Appendix J

Laboratory Exercise: Determining Activation of T Cells with an ELISA

Objectives:

- To determine if T cell activation has occurred by running an ELISA to test for the presence of IL-2 in a sample
- Based on the ELISA results, determine if a new experimental drug is effective at suppressing the activation of T-cells in an animal model of MS

Background:

Neuropathic pain is pain that is felt even without the stimuli that would normally be detected by the peripheral nervous system. This type of pain usually occurs as a result of nerve injury or disease (Schomberg & Olson, 2012). Chronic neuropathic pain can be caused by a number of factors, from cancer, autoimmune diseases, and neurodegenerative diseases such as multiple sclerosis (MS). Current treatments for chronic neuropathic pain are not very effective and have uncomfortable or even dangerous side effects; more research needs to be done to find new, more effective treatments. One focus of research into new treatments is the inhibition of effector T-cells and glial cell activation (Murphy, Bethea, & Fischer, 2017).

Microglial cells, a type of glial cell in the central nervous system, have been shown to play a role in the development of neuropathic pain. These cells are similar to macrophages, and they have the ability to activate both innate and adaptive immune responses. When activated, microglial cells can release cytokines and chemokines which attract nearby innate immune cells and can also produce antigen presenting molecules which interact with T-cells (Schomberg & Olson, 2012).

In this activity, you are a researcher working with the pharmacology department to develop drugs that work to inhibit the immune response and hopefully find a drug that will diminish the pain symptoms of MS patients with chronic neuropathic pain. Your lab works with mice models with experimental autoimmune encephalomyelitis. These mice have a disease similar to MS, and they are commonly used in pain research (Murphy et al., 2017). You have isolated several samples from your mice. These samples were taken from the spinal cord and dorsal root ganglion. One set of samples have been given an experimental compound which is designed to inhibit the activation of T-cells, the other set are the control samples. The T-cells present in the samples have been isolated and cultured. It is your job to determine the extent of T-cell activation in each sample. You will do this by performing an ELISA which will determine the presence of IL-2, produced by activated T-cells.

Works Cited

Murphy, K. L., Bethea, J. R., & Fischer, R. (2017). Neuropathic pain in multiple sclerosis: Current therapeutic intervention and future treatment perspectives. In I. S. Zagon & P. J. McLaughlin (Authors), *Multiple sclerosis: Perspectives in treatment and pathogenesis* (pp. 53-69). Brisbane, Australia: Codon.

Schomberg, D., & Olson, J. K. (2012). Immune Responses of microglia in the spinal cord: Contribution to pain states. *Experimental Neurology*, *234*(2), 262-270.

Materials:

- 4 yellow tubes (test samples 1-4)
- 1 purple tube (positive control)
- 1 blue tube (negative control)
- 1 green tube (primary antibody **PA**)
- 1 orange tube (secondary antibody **SA**)
- 1 brown tube (substrate **SUB**)
- 2 12-well microplate strips
- 20-200 µl adjustable micropipet (or a 50 µl fixed-volume micropipet)
- micropipet tips

- 1 disposable transfer pipet
- 70-80 ml was buffer (in beaker)
- 1 Sharpie
- paper towels

Procedure*:

1. Label each well of your 12-well strip. Each sample will have 3 wells.
    a. positive control (+)
    b. negative control (-)
    c. test samples (label each well with the number on the sample tube)
2. Using a clean, unused pipet tip, transfer 50 µl of the positive control into the three wells labeled "+".
3. Discard pipet tip.
4. Using a new pipet tip, transfer 50 µl of the negative control into the three wells labeled "-".
5. Discard pipet tip.
6. Using a new tip for each sample, transfer 50 µl of the test samples into their correct wells. Make sure to use a new tip for each sample.
7. Wait 5 minutes to allow the antigens to bind to the plastic walls of the wells.
8. Wash out the wells by following the steps below:
    a. Turn the microplate strip upside down on top of the paper towels and gently tap to remove the liquid. Be careful not to splash the samples back into the wells or into other wells.
    b. Discard the paper towels.
    c. Fill each well with wash buffer, but be careful not to overfill the well. This could cross-contaminate the neighboring wells.
    d. Turn the microplate strip upside down again on another paper towel and tap gently.
    e. Discard the paper towels.
9. Repeat step 8 (washing step).
10. Using a new pipet tip, transfer 50 µl of the primary antibody into all of the wells.
11. Wait 5 minutes for the antibody to bind to the antigens in the samples.
12. Repeat step 8 (washing step) **two times** to remove any unbound antibody.
13. Using a new pipet tip, transfer 50 µl of the secondary antibody into all of the wells.
14. Wait 5 minutes for the antibodies to bind.
15. Repeat step 8 (washing step) **three times** to remove any unbound antibody.
16. Using a new pipet tip, transfer 50 µl of the enzyme substrate (SUB) into all of the wells.
17. Wait 5 minutes. Observe and record the color change in the data table. Record a "+" if the sample turned blue and a "-" if there was no color change.
18. Take a photo of your wells against a white background.

*Adapted from Bio-Rad Laboratories, Inc. (n.d.). *Biotechnology Explorer ELISA Immuno Explorer Kit Instruction Manual*. Hercules, California: Bio-Rad Life Science Group.

Data:

In the table below, indicate whether or not there was a change in color in each of the wells. Indicate a change in color with a "+" and no change with a "–". Remember you should have 3 wells for each sample.

Sample	Well 1	Well 2	Well 3
Positive Control			
Negative Control			
Test Sample #1 (dorsal root, experimental)			
Test Sample #2 (spinal cord, experimental)			
Test Sample #3 (dorsal root, control)			
Test Sample #4 (spinal cord, control)			

Analysis Questions:

1. What is the importance of the positive and negative control samples?

2. Why do you use 3 wells for each sample? Be specific in your explanation.

3. Explain how the ELISA test detects IL-2 in the sample.

4. What do your results tell you about the activation of T-cells in each sample? Be specific in your response.

5. How do the colors of your positive test samples compare to that of the positive control? What does that mean about the proliferation of T-cells in your samples?

6. Based on your results, what can you determine about the effectiveness of the experimental drug?

Appendix K
T Cell Activation Lab Report Outline

General Requirements
Your lab report must include/follow these requirements:

- written in 3rd person (no I, me, we, us, etc.)…steer clear of using "the group"…the only time you should talk about a person is when you discuss test subjects
- double-spaced, Times Roman, 12 pt. font
- grammar and spelling will be graded
- include each section heading

Title Page
Include a title page following the format below. Your title needs to be factual and descriptive (it shouldn't be "T Cell Activation Lab").

Your title on the first line
Date the experiment was performed
Your name
Lab partners names

Purpose
Explain the purpose of this experiment. What were you trying to determine? Why might this research be important in real life?

Theory
Include background information on your experiment. What is already known about what you are testing? Make sure to define words that the reader needs to know in order to understand the results (example…explain how T cells are activated). Include information from ***at least two reliable sources***. Make sure you cite your sources in the text in MLA format and include them in a Works Cited page at the end of your report. Include your hypothesis statement at the end of this paragraph.
*****A note on sources…I will not accept dictionaries as sources of information for this report. The textbook would be a great source of information. If you need help finding sources please let me know.*****

Method
Write your materials needed for this experiment and the procedure you followed. This should be written in paragraph form. Make sure it is easy to follow and can be replicated by another person if they wanted to do the exact same experiment. Do not list your materials and then the procedure; explain what you did and the materials will be included along the way.

Example (osmosis egg lab from freshman biology)…the materials are underlined for demonstration purposes:

Two eggs were soaked in vinegar for 24 hours to dissolve the shells. The eggs were then washed to remove any remaining shell residue. The eggs were weighed using a digital scale. A cup was placed on the scale and the scaled was zeroed to remove the weight of the cup. Then the egg was placed in the cup and the initial mass was recorded. This was repeated for the second egg.

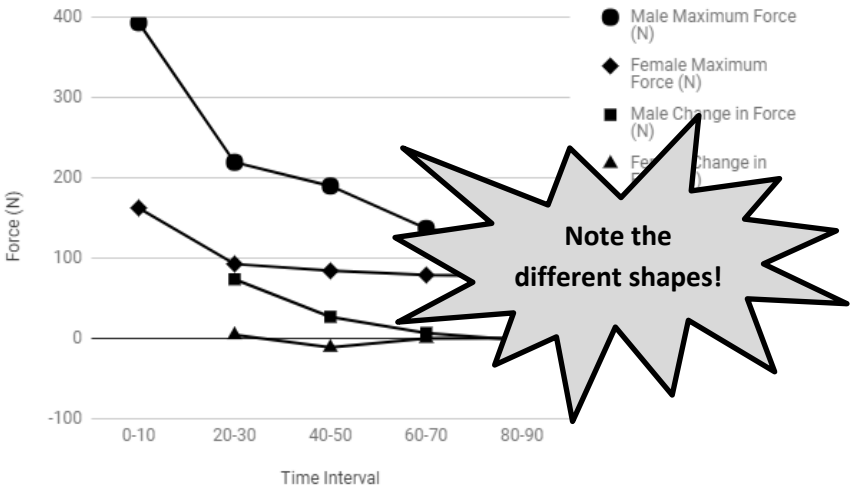
Each egg was placed in a cup, one cup for corn syrup and one for distilled water. Corn syrup and distilled water was added to the cups, just enough to cover the eggs. After 24 hours, the eggs were removed from the cups and weighed again, following the same procedure above. The final mass was recorded and the change in mass was calculated using the formula below:

Final Mass – Initial Mass = Change in Mass

Data and Results

You will be deciding how to best represent your data. This could be in the form of a table, or a table and graph. Reminders about tables and graphs:

- **<u>Titles for tables go above, graphs below.</u>**
- Each title starts with either Table 1. or Figure 1. and the number may change depending on how many you have.
- Titles need to be factual and descriptive. I should be able to determine what I will learn from the table/figure from your title.
- <u>Make sure to include units!</u>
- Unless you are printing in color, you should use shapes to differentiate the data points.
    - If you are making a bar graph, make sure to change the color of the bars to grayscale unless you print in color.



Discussion

Your discussion should have three separate paragraphs:

Paragraph 1 – Data
Explain your data in this paragraph, do not make any conclusions about what your data means. Make sure to reference your tables and graphs in this paragraph when you talk about the data (ex: "As shown in Table 1 and Figure 1, the male subject applying continuous force had the highest maximum grip force of the four experiments…"). Talk about **<u>trends in the data, not every single data point collected.</u>**

Paragraph 2 – Support/Reject Hypothesis
In this paragraph include the following information:

- restate your hypothesis
- say whether your data supported or rejected your hypothesis
- explain how the data supports or rejects your hypothesis
- make inferences about what the data means, this should connect back to what you discussed in the theory

Paragraph 3 – Limitations and Sources of Error
In this paragraph explain at least three limitations and/or sources of error for this experiment. Human or equipment error does not count. If you say the equipment is not accurate enough, you need to provide a better alternative (example: instead of using a ruler to measure you can use a caliper which would be more accurate). Give possible solutions for each of these.

T Cell Activation Lab Report Rubric

Category	Points Earned
Title Page: (5 points) • title is included and is factual and describes the experiment ___/3 • date and lab partners names are included ___/2	
Introduction (Purpose/Theory): (5 points) • purpose of lab is stated, connects to real-life ___/2 • theory includes background information __/2 • hypothesis statement is included ___/1	
Method: (10 points) • written in paragraph form ___/2 • all materials needed are listed ___/3 • steps are clear and concise, anyone could replicate the experiment ___/5	
Data and Results: (15 points) • format (tables before graphs, titles in proper locations) ___/5 • tables included with appropriate units and a descriptive title ___/4 • figures included with appropriate units and a descriptive title ___/4 • tables/graphs effectively present the data (appropriate graphs, etc.) ___/2	
Discussion: (10 points) • data is described ___/1 • hypothesis is restated ___/1 • hypothesis is supported or rejected using the data ___/2 • inferences are made to connect the results to the theory ___/3 • three limitations or sources of error are suggested with possible solutions ___/3	

Format: (15 points)
• grammar (complete sentences, punctuation, word choice, spelling) ___/5
• organization/formatting of sections ___/1
• no 1st person ___ /1
• report is double-spaced, in 12 pt. Times Roman font ___/2
• citations:
  ○ works cited page included in MLA format with 2 reliable sources ___/3
  ○ in-text citations included and in correct format ___/3	

Total Points Earned: _____ / 60	