AAI High School Teachers Summer Research Program in Immunology: Primer on Immunology using Dengue Fever as a Real World Application. Extension: Detection of the Prevalence of Lyme Disease in Local Canine Populations.

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I. Science Background

Primer of Immunology

Brief Review of the Immune System

The immune system of animals is made up of two defense mechanisms; the Innate and Adaptive Immune Systems.

A) Innate Immune Response - Nonspecific defenses that attempt to prevent pathogens from entering the organism or quickly eliminate pathogens that do enter the organism.

1) First line of defense – barriers that prevent the pathogen from entering the organism.
   i. Skin
      Presence of normal bacteria and fungi that out-compete pathogens
      Acidic pH
      Skin Saltiness
   ii. Mucus membranes trap pathogens.
      Lysozyme- enzyme made by mucus membranes, it breaks open cell walls.
      Defensins – peptides made by mucus membranes that insert themselves into plasma membranes making them freely permeable to water.

2) Second line of defense is initiated when pathogens breach the skin or mucus membranes and gain access to the organism.
   i. Here pathogens encounter phagocytes, natural killer cells and defensive molecules such as complement and interferon proteins.
   ii. Inflammation stops the spread of tissue damage; it recruits molecules and other cells to the injury site to eliminate pathogens and promote tissue repair.
   iii. Mast cells, one of the first cells to respond to tissue damage, release several different chemicals to aid in the repair process.
      - Tumor necrosis factor is a cytokine that kills target cells.
      - Prostaglandins play a role in initiating the inflammatory process.
      - Histamine increases blood vessel permeability to allow white blood cells and molecules access to affected tissues.

B) Adaptive Immune Response – Is initiated when the body detects the presence of an antigen. It has four key features:

i. Specificity – Each T cell and B cell is specific for one antigen. When an antigen (from a pathogen) binds to a T cell receptor and an antibody is produced by a B cell, a specific immune response is started.

ii. Diversity – To respond to the variety of pathogens the body produces many different lymphocytes, each bearing a unique antigen receptor. This is accomplished through DNA mutations of receptor proteins during cell formation in the bone marrow. It also occurs

when a T or B cell is activated through antigen binding causing it to divide to form clones (genetically identical cells). This is called clonal selection.

iii. Distinguishing Self from Nonself – Through clonal deletion B or T cells that have the potential of attacking one's own body cells are eliminated through apoptosis.

iv. Immunological Memory – Once the immune system responds to a specific pathogen the system "remembers" this pathogen and can respond rapidly the next time it is found.

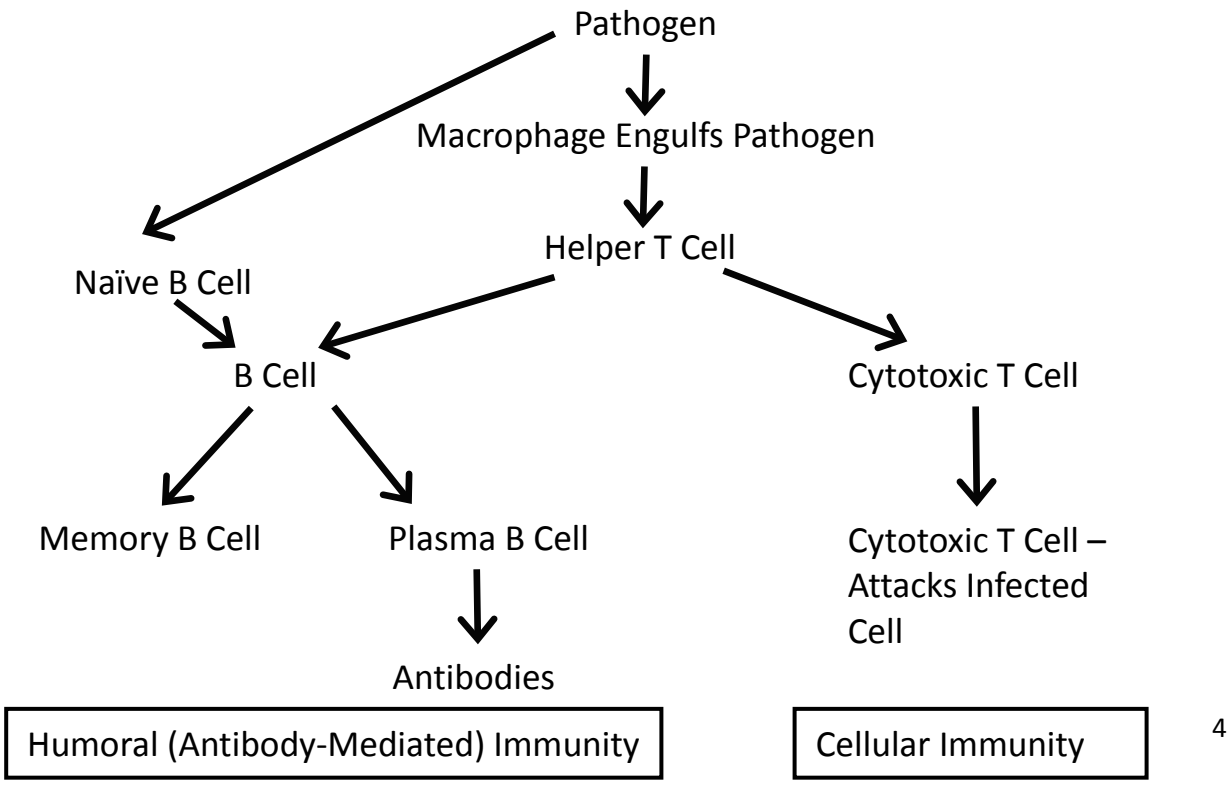
1) The primary immune response can take several days before B cells produce antibodies and T cells are produced with the appropriate receptors. When the B and T cells divide they make two different types of cells, effector and memory.

Effector cells – Effector B cells known as plasma B cells secrete antibodies. Effector T cells (helper T cells and cytotoxic T cells) secrete cytokines and other molecules that destroy nonself or infected cells.
Memory Cells – Memory B and T cells are long lived and can divide on short notice to produce more memory and effector cells.

2) Secondary immune response occurs after the primary response to a specific antigen. Memory cells that bind to the antigen reproduce to form plasma B cells and T cells.

C) Humoral vs Cellular immune response – B cells are primarily involved with the humoral response and helper and cytotoxic T cells with the cellular response.

<u>Flow chart of Adaptive Immune Response</u>



There are five classes of antibodies secreted by B cells.

IgM – the first class of antibodies produced after the initial exposure to an antigen and is short lived.

IgG – is the most abundant antibody in blood and tissue fluids.

IgD – acts as cell surface receptor on B cells.

IgA – provides protection for mucus membranes.

IgE – present in blood, triggers release of histamines and other chemicals involved in allergic reactions.

Dengue Q & A

Question: What is dengue?
Answer: Dengue is a vector borne viral disease caused by one of four different serotypes (DENV-1, DENV-2, DENV-3, DENV-4). The virus is a member of the Flaviviridae family.

Question: How is it contracted/transmitted?
Answer: The most common vector is the *Ades aegypti* mosquito. *Ades albopictus* is also a carrier. When an infected mosquito bites a human, the virus can be transmitted through the fluids the mosquito inserts through its proboscis. The mosquito acquires the virus through the blood of an infected human it bites. Human to human transmission is not possible.

Question: Can I get dengue again if I have already been infected?
Answer: You will have immunity to the specific virus (DENV-1, DENV-2, DENV-3, DENV-4) that you were originally infected with and, if infected again with that serotype, you will not get the disease. However, if you are infected with a different serotype you are at a greater risk of acquiring dengue hemorrhagic fever (DHF). DHF is a more severe form of dengue and can be fatal if medical treatment is not immediately sought.

Question: How can it be prevented?
Answer: Because the disease is spread by mosquitoes, eradication of breeding sites is essential. *Ae. aegypti* lays her eggs in containers that hold water (e.g. tires, planters, water drums). However, *Ae. aegypti* are adaptable to varying conditions; eggs have been known to survive drought conditions. *Ae. albopictus* prefers cool dark areas such as home closets. The use of air conditioning and screens can prevent adults from finding their human meals.

Question: What are the disease symptoms?
Answer: Many of the symptoms are very similar to malaria or the flu, they include: high fever; severe headache; pain in the joints, muscle, bone or behind the eyes; rash; and mild bleeding. The symptoms of dengue hemorrhagic fever include a fever that lasts from two to seven days with the other mentioned symptoms. As the fever declines the following symptoms may occur: vomiting, abdominal pain and breathing difficulties. This opens a 24 to 48 hour period when capillaries start to leak fluid. This increase in fluid in the body cavities can lead to the failure of the circulatory system, shock, and death. Skin hemorrhages, along with internal and external bleeding, are also possible.

Question: How is dengue treated?
Answer: There is no specific treatment for the disease. If one suspects they have dengue fever, they can take analgesics, stay hydrated, rest, and speak with their doctor. If they develop more severe symptoms after the fever has subsided, they should go to a hospital. Fluid replacement therapy is appropriate at this point. Currently, there is no vaccine on the market; however the company Sanofi Pasteur has plans to register the tetravalent vaccine (protects against all 4 dengue strains) in 2015 with a target distribution time frame of late 2015.

Questions: Where has the disease been found?
Answer: The four viruses originated in monkeys and spread to humans in Africa or Southeast Asia between 100 and 800 years ago. Currently, the disease can be found in Southeast Asia, the Pacific Islands, Africa, Caribbean, and the Americas.

Question: What lab tests are used to detect the virus?
Answer: The test methods used is dependent upon the duration of the disease.

Real time RT-PCR is used to detect the virus in patients within the first 5 days of symptoms. A positive result confirms presence of the disease and usually which viral strain is involved. A negative result in this time frame is termed indeterminate, and patients will be asked for another sample 5 days post symptoms.

MAC ELISA detects the presence of IgM antibody in the serum. The test can be used as early as 3 days post symptoms and may be able to detect the antibody as far as 90 days post symptoms. Caution should be used interpreting the results as this method can also react with other flaviviruses.

IgG ELISA is used to diagnose the presence of a past infection. To determine if the infection was primary (first exposure to dengue) or secondary (a second exposure) in nature, two samples need to be taken, one during the acute phase and one in the recovery phase. If the results are negative in the acute but positive in the convalescent phase, the patient has a primary infection. If there is a positive result in the acute phase and a fourfold increase in the convalescent phase, the infection is secondary.

Plaque Reduction and Neutralization Test is used to detect specific serotypes in the convalescent serum.

Glossary of Basic Immunology Terms

Antibody – proteins that bind to specific substances (antigens) on “non-self” cells. They are made by plasma cells in response to a pathogenic attack.

Antigen – a molecule usually bound to a pathogen that stimulates the production of a specific antibody on B cells and cell division in T and B cells.

B Cells (B lymphocytes) – have receptors that are specific for one antigen type. When bound to an antigen they will differentiate into B cells that produce antibodies for that specific antigen.

Complement Proteins - a set of diverse proteins that function in a cascade manner. One complement protein binds to proteins on the pathogen which alerts phagocytes to the presence of an invader. Another protein activates the inflammatory response. Other proteins are responsible for lysing the pathogen.

Cytokines – signaling proteins that affect the behavior of their target cell.

Immunity – the body's ability to resist disease when invaded by a pathogen.

Inflammation – damaged tissue that exhibits puffiness, warmth, and redness.

Interferon – a class of cytokines produced by virally infected cells. This signal protein alerts neighboring cells increasing their ability to resist infection.

Lymphocyte – type of white blood cell that is in involved in adaptive immunity; it includes B cells and T cells.

Major Histocompatibility complexes (MHC) – proteins found on a host cell's surface that hold an antigen fragment that can bind to a T cell receptor.

Natural Killer Cells – a type of lymphocyte that recognizes cells that are infected or cancerous and initiates cell destruction. They are part of the innate immune system.

Pathogen - organisms and viruses that can cause disease when they infect a host.

Phagocyte – white blood cells that engulf pathogens, they are involved in innate and adaptive immunity (e.g. macrophage).

T cells (T lymphocytes) –mature in the thymus. They have a variety of functions: activating macrophages, helping B cells produce antibodies, and killing infected cells.

T cell receptors – proteins found bound in the membrane of T cells. They recognize and bind to non-self substances (antigens) bound to MHC proteins on other cells.

II. Student Outcomes

Concepts Covered:

Students will have a deeper understanding of the adaptive immune system. Through the use of virtual labs and videos, students will be familiarized with the techniques of ELISA, PCR, RT-PCR, and flow cytometry. By completing the dengue case study, students will learn about the cause, symptoms, diagnosis, and treatment of dengue fever and dengue hemorrhagic fever.

Standards Addressed:

AP Biology

- Essential knowledge 2.D.4 – Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.
- Essential knowledge 3.D.2 – Cells communicate with each other through direct contact with other cells or from a distance via chemical signaling.

Next Generation Science Standards

- HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
- HS-LS1-3. Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

Placement in Course
These lessons can be incorporated in an Immunology unit or Biotechnology unit. The lessons are designed for an Honors Biology and/or AP Biology course.

What students will do and technical skills learned
Students will review/learn the basics of the adaptive immune system. Through the use of cell images, students will reinforce their knowledge of the various forms of B and T cells and how they interrelate. Students will complete a virtual ELISA and PCR lab to gain knowledge of those processes along with becoming familiar with flow cytometry. By completing the dengue fever case study, students will learn about the etiology of the disease, and how to diagnosis the disease via RT-PCR, MAC and IgG ELISA.

III. Learning Objectives
AP Biology–
- LO 2.29 The student can create representations and models to describe immune responses.
- LO 2.30 The student can create representations or models to describe nonspecific/specific immune defenses in plants and animals.
- LO 3.34 The student is able to construct explanations of cell communication through cell to cell direct contact or through chemical signaling.
- LO 3.35 The student is able to create representations that depict how cell to cell communication occurs by direct contact or from a distance through chemical signaling.

IV. Time Requirements
This unit can be completed in three to four 50-minute blocks. Day 2 may require 2 blocks of time depending in the student's computer familiarity and the number of techniques assigned.

V. Advance Preparation
Copying Student handouts
Reproduce cell images (Note: Laminate the copies for repeated use.)

VI. Materials and Equipment
- Cell images – laminated or on card stock for use with multiple classes over multiple years. One set per student.
- Handouts for technology. One set per student.
- Computer access. One computer per student if possible.
- Dengue fever Case Study. One set per student.

VII. Student Prior Knowledge.
Students should have a basic knowledge of the function of the human immune system. They should have the ability to successfully navigate virtual labs.

VIII. Daily Unit Plans

Daily Lesson Plans

General overview of unit - The unit is designed to introduce or reinforce the concepts of the adaptive immune system to students using dengue fever as a real-life application.

Assign the video and reflection guide (see Student Section) to be completed prior to the start of the unit.
http://www.bozemanscience.com/immune-system

Day 1 – Teacher will review or introduce (depending on the class level) the components of the adaptive immune system; how different cell types interrelate and the general schemata of how the human body responds to a pathogenic insult.

> *Activity* -could be used Day 1 as reinforcement or Day 2 as formative assessment. Each student will be given a set of images that contain the different cell types of the adaptive immune system. The images are located in the Student Section. Students will first be asked to separate them into different cell types (e.g. B and T cells). Then, using the cell images students will create a flow chart representing how the immune system responds to an exposure to a pathogen for the first time. Next, they will arrange the cells as if this is the second exposure to the pathogen. This is an individual activity; each student will do this independently.

Day 2 – Students will be introduced to the techniques used in the immunology, medicine, and/or research arenas. Each student should have access to a computer and the internet.

> *Activities that could be used include:*
>
> a) ELISA
>
> This simulation guides the students through the ELISA technique from set-up through data analysis. The Immunology Virtual lab simulation and student worksheet from HHMI are located here:
>
> http://www.hhmi.org/biointeractive/immunology-virtual-lab
>
> b) PCR
>
> This short video on PCR (1:27 minutes) from the DNA Learning Center at Cold Spring Harbor briefly describes the process of PCR (Polymerase Chain Reaction).
>
> https://www.youtube.com/watch?v=2KoLnIwoZKU
>
> The associated worksheet is located in the Student Section and the answers are found in the Appendix.
>
> This virtual lab takes students through the laboratory experience of performing a PCR.
>
> http://learn.genetics.utah.edu/content/labs/pcr/
>
> The associated worksheet is located in the Student Section and the answers are found in the Appendix.

c) Reverse Transcription PCR
This is a general written overview of the RT-PCR technique from Davidson College.
http://www.bio.davidson.edu/people/Kabernd/seminar/2002/method/lowry/RTPCR.htm
The associated worksheet is located in the Student Section and the answers are found in the Appendix.

d) Flow Cytometry
A basic introduction to the technique of Flow Cytometry.
http://www.biotechnologyforums.com/thread-2185.html
The associated worksheet is located in the Student Section and the answers are found in the Appendix.

Additional Resources that may be of use:

ELISA

- MAC ELISA step by step directions. This is highly detailed information suitable for accelerated AP Biology students.
  http://www.diatek.in/inbios/DENV%20Detect%20IgM%20ELISA.pdf
- IgM CAPTURE ELISA step by step directions. This is highly detailed information suitable for accelerated AP Biology students.
  http://www.inbios.com/cms/file/900106-04%20IVD%20DENV%20Detect%20IgM%20Capture%20ELISA%20insert%281%29.pdf

RT-PCR

- This animation steps the viewer through the basic steps of RT-PCR.
  http://www.bio.davidson.edu/courses/immunology/flash/rt_pcr.html
  There are no associated written assignments with this animation.

DAY 3 – Dengue Fever Case Study
Students will work in small groups of 2-3.
The case study focuses on the principles of immunology as told through a person who may have been bitten by a mosquito carrying the dengue virus. Students will act as the health care worker trying to determine if the person has contracted dengue fever. Questions will be interspersed throughout the case study.

Additional Resources Associated with Dengue:

- *General Information*

World Health Organization. Dengue and severe Dengue (2013).
Centers for Disease Control. Dengue (2013).
Nature. http://www.nature.com/scitable/topicpage/current-dengue-fever-research-22404441
You Tube. https://www.youtube.com/watch?v=0PUsdv1kDTc
The New York Times. *http://www.nytimes.com/health/guides/disease/dengue-hemorrhagic-fever/overview.html*

- *Prevention*

This Blog discusses the use of genetically modified mosquitoes to control their population size.
http://www.nature.com/scitable/blog/viruses101/are_modified_mosquitoes_the_future

Campbell discusses the world wide spread of dengue and current attempts to prevent the spread.
Campbell, C. "If You're Not Worried About Dengue Fever, Here's Why You Should Be." *Time*. November 18, 2013.

IX. How will students demonstrate understanding?

- Successful arrangement of cell images according to directions.
- Successfully answering the questions regarding technology and completion of the virtual labs.
- Successful completion of the case study.

X. References

Clinic Staff, Mayo. "Dengue Fever." Mayo Clinic. N.p., 19 Sept. 2014. Web. <http://www.mayoclinic.org/diseases-conditions/dengue-fever/basics/definition/con-20032868>.

"Dengue." Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, 26 Mar. 2015. Web. <http://www.cdc.gov/Dengue/>.

"Dengue Guidelines for Diagnosis, Treatment, Prevention and Control." (2009): 1-160. Dengue. World Health Organization, 2009. Web. <http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf>.

DeSalle, Rob, and Michael R. Heithaus. *Holt Biology*. Orlando, Fla: Holt, Rhinehart and Winston, 2008. Print

Garcia-Navarro, Lourdes. "Ready, Set, Spray! Brazil Battles Dengue Ahead Of The World Cup." NPR. NPR, 29 May 2014. Web. <http://www.npr.org/blogs/health/2014/05/29/316668643/ready-set-spray-brazil-battles-dengue-ahead-of-the-world-cup>.

Hillis, David M. *Principles of Life*. Sunderland, MA: Sinauer Associates, 2012. Print

Murphy, Kenneth, Paul Travers, Mark Walport, and Charles Janeway. *Janeway's Immunobiology*. New York: Garland Science, 2008. Print.

Reece, Jane B, and Neil A. Campbell. *Biology*. Boston: Pearson Learning Solutions, 2008. Print.

XI. STUDENT SECTION

This section contains:

- Video Reflection Guide
- Immune Cell Images
- What are PCR, RT-PCR and Flow Cytometry?
- Case Study – Dengue Fever

Video Reflection Guide – Immune System

Name_____ Block_____

Respond to the following questions after watching the Immune System Video.
http://www.bozemanscience.com/immune-system

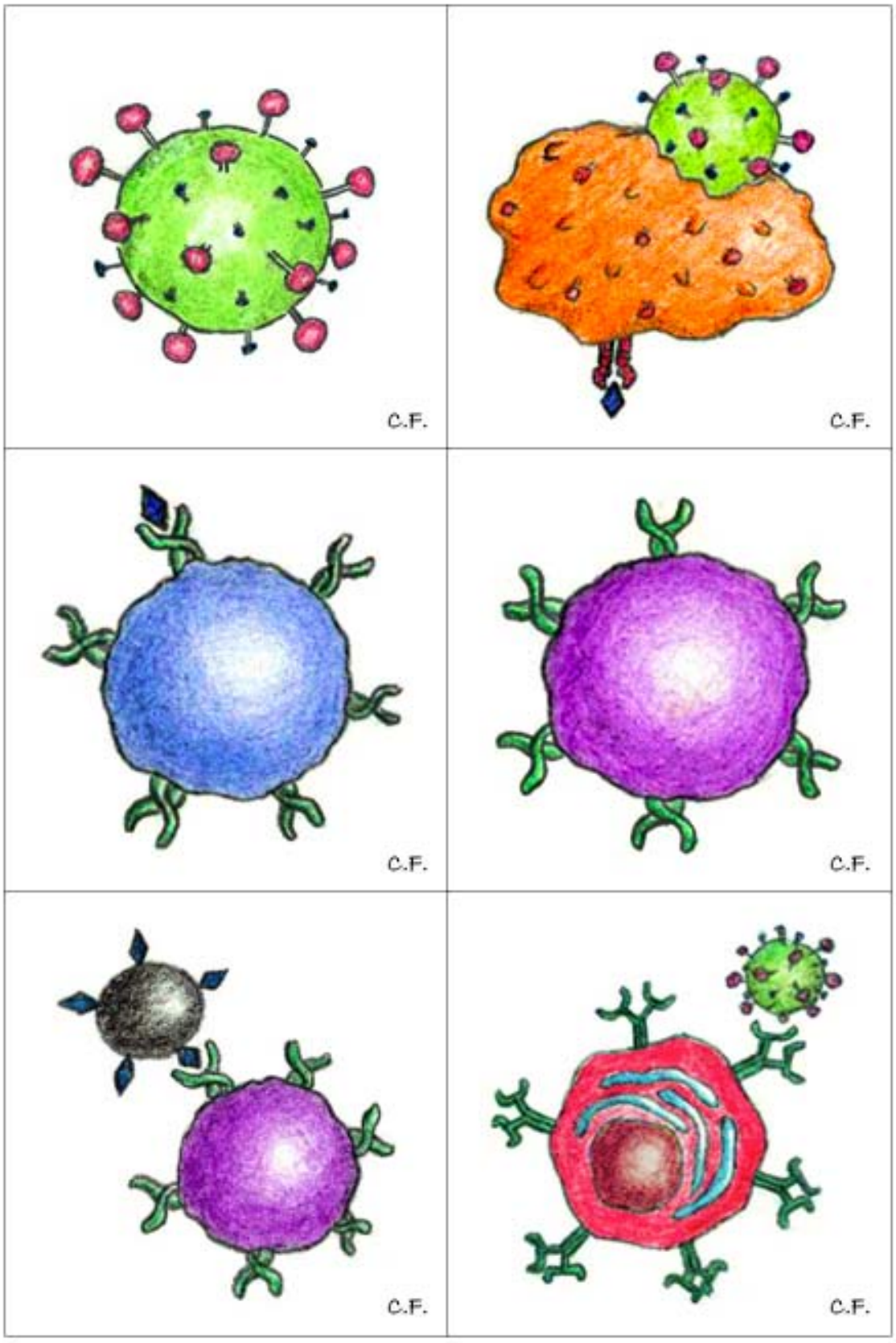
Brief Summary **"In this video presentation I learned…"**

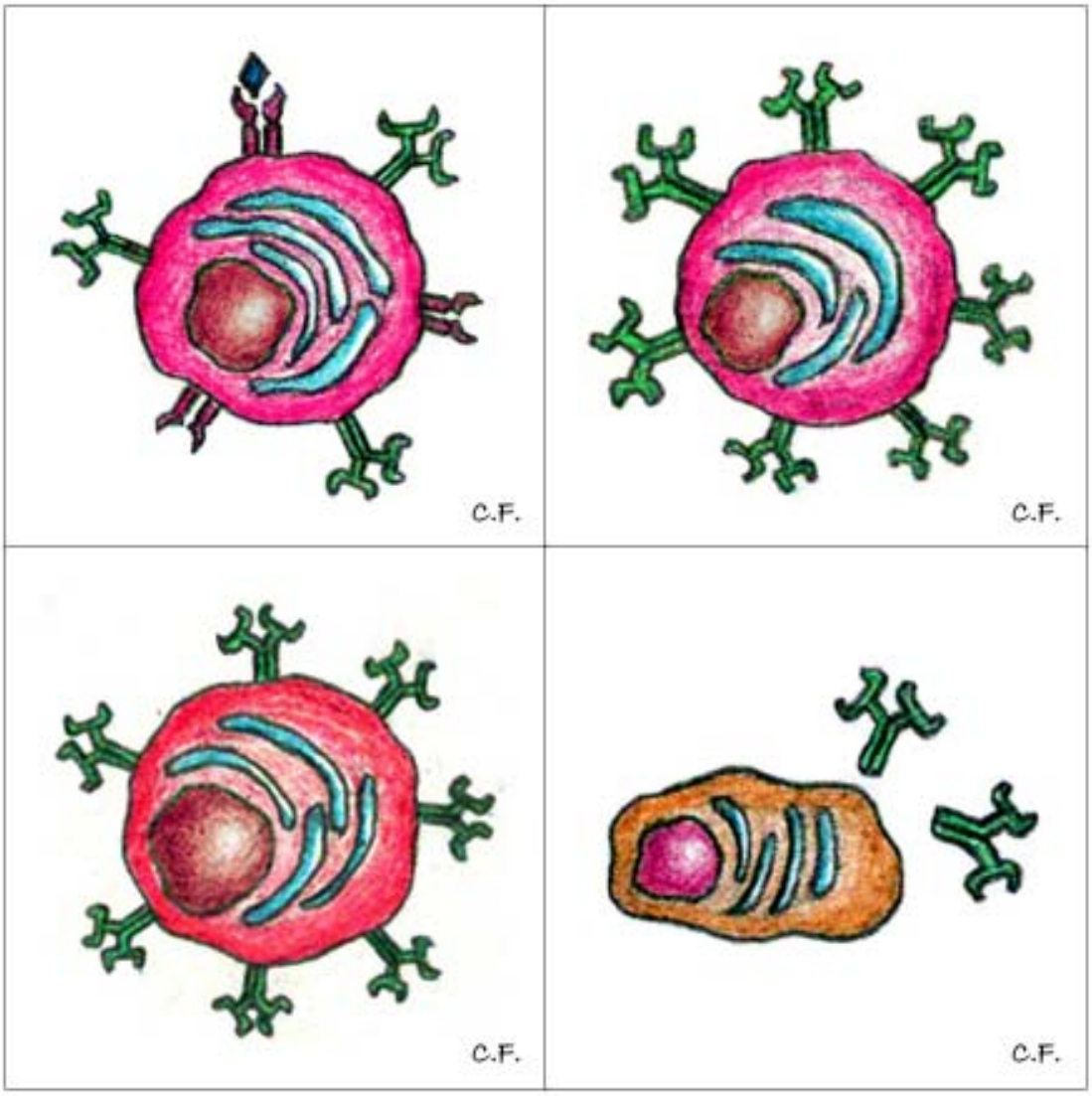
The main points **"Specifically…" (bullet points are appropriate here)**

What you did not understand well **"I am still confused about…"**

Optional
"I was really excited to learn about…." OR "I would like to learn more about…"

Innate and Adaptive Cells of the Immune system

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What are PCR, RT-PCR and Flow Cytometry?

Name_____ Block_____

I. The following video from the DNA Learning Center at Cold Spring Harbor, explains the steps that take place in the typical PCR reaction.

https://www.youtube.com/watch?v=2KoLnIwoZKU

1) PCR uses repeated cycles of what to make many copies of DNA?

2) What causes the DNA to denature into single strands?

3) What is added to the DNA once the temperature has been decreased and then been raised slightly?

4) What is the function of the primer and Taq Polymerase?

5) How many cycles are needed before the desired DNA sequence starts to accumulate?

II. Virtual PCR Lab from Learn.Genetics the Genetics Science Learning Center at University of Utah Health Sciences.

http://learn.genetics.utah.edu/content/labs/pcr/

Define the following vocabulary words located on the home page.

Primer –

DNA Polymerase –

Nucleotide –

Continue on to the PCR Virtual lab and answer the following questions.

1) What is the importance of PCR?

2) Where can DNA samples come from for PCR reactions?

3) To start a PCR reaction what do you need to add to the PCR tube besides the DNA?

4) What is the name of the machine that can heat and cool the PCR tube?

5) What happens to DNA at 95^0C?

6) Once the DNA Polymerase locates the primers what does it do?

7) By the end of cycle three, how many copies of the DNA fragment you targeted are there?

III. You now have an understanding of the basic principles of PCR. Read the following short article on Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and answer the following questions.

http://www.bio.davidson.edu/people/Kabernd/seminar/2002/method/lowry/RTPCR.htm

1) What nucleic acid is detected by this technique?

2) If that nucleic acid is present in the sample being tested, what does it mean for a specific gene that it is associated with and its product?

3) What are the steps in the "RT" portion of RT-PCR?

IV. What is Flow Cytometry?

Read the information on the Flow Cytometry and answer the accompanying questions.
http://www.biotechnologyforums.com/thread-2185.html

1) What is the function of a flow cytometer?

2) What is the basis for the separation of different cell types?

3) Explain two uses for this technique.

Dengue - Is the World Cup Worth the Risk?

Introduction:

October 30, 2007 – Brazil has been elected as the host nation for the 2014 FIFA World Cup. Fast forward to August 20, 2013.

Chris J., an enthusiastic soccer fan, is anxiously awaiting the online ticket sales of the 2014 FIFA World Cup to open up. Chris is a Ph.D. student in the Microbiology and Immunology Department at Upstate Medical University. He spends his summers in Brazil researching the mosquito dengue fever vectors, *Aedes aegypti and Aedes albopictus.* Next summer, while collecting mosquitoes from the City of Natal, he is hoping to see the U.S. play a soccer match in the Arena das Dunas. He will be taking Drew, an undergraduate research assistant, with him to help with collecting the mosquitos and he wants to go to the match as well.

As their June departure date draws closer, Drew excitedly approaches Chris. "Chris I heard a story on NPR this morning about dengue fever and how Brazil is having an epidemic this year. Here is the transcript of the interview."

Ready, Set, Spray! Brazil Battles Dengue Ahead Of The World Cup

Transcript: http://www.npr.org/templates/transcript/transcript.php?storyId=316668643

Drew: "In another news article it said that the cities of Natal, Fortaleza, and Recife have the greatest risk of dengue fever. We are headed to Natal. I'm nervous that I will be bitten by a mosquito carrying one of the dengue viruses?"

Answer these questions based on the NPR interview.

1) How is the dengue infection spread?

2) How many different strains of dengue are there?

3) How is poor water infrastructure contributing to the spread of dengue?

4) If you were Drew would you be concerned about contracting dengue fever? Support your answer.

Background

Chris: "While I can't guarantee that you will not be exposed to dengue while we are in Brazil, here is what you need to know about the disease and its transmission."

Question: What is dengue?
Answer: Dengue is a vector borne viral disease caused by one of four different serotypes (DENV-1, DENV-2, DENV-3, DENV-4). The virus is a member of the Flaviviridae family.

Question: How is it contracted/transmitted?
Answer: The most common vector is the *Ades aegypti* mosquito, *Ades albopictus* is also a carrier. When an infected mosquito bites a human, the virus can be transmitted through the fluids the mosquito inserts through its proboscis. The mosquito acquires the virus through the blood of an infected human it bites. Human to human transmission is not possible.

Question: Can I get dengue again if I have already been infected once?
Answer: You will have immunity to the specific virus (DENV-1, DENV-2, DENV-3, DENV-4) that you were originally infected with and, if infected again with that serotype, you will not get the disease. However, if you are infected with a different serotype you are at a greater risk of acquiring dengue hemorrhagic fever (DHF). DHF is a more severe form of dengue and can be fatal if medical treatment is not immediately sought.

Question: How can it be prevented?
Answer: As the disease is spread by mosquitoes, eradication of breeding sites is essential. *Ae. aegypti* lays her eggs in containers that hold water (e.g. tires, planters, water drums). However, *Ae. aegypti* are highly adaptable to varying conditions; eggs have been known to survive drought conditions. *Ae. albopictus* prefers cool dark areas such as home closets. The use of air conditioning and screens can prevent adults from finding their human meals.

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Plaque Reduction and Neutralization Test is used to detect specific serotypes in convalescent serum.

Drew: "Thank you Chris for all of that information. I feel better about my chances of contracting dengue now."

Answer the following questions based on the above information.
6) What are the symptoms of dengue fever and dengue hemorrhagic fever?

7) What tests can be used to confirm the disease?

The story continues: Chris and Drew travel to Brazil

Chris and Drew organize the supplies needed for their research in Natal, Brazil. Mindful of the continued news articles about the threat of dengue, they pack appropriate clothing and repellents to deter mosquitoes. On June 1, 2014, they leave Syracuse for Brazil.

Their research went well; they were able to sample adult mosquito populations as well as collecting eggs from a variety of sources. While it was winter in Brazil mosquitoes were still plentiful. On June 16th, the day before they were to leave Natal, they went to the Arena das Dunas to watch the US vs Ghana soccer match. The US won 2-1, what a great way to end their trip!

Symptoms?

Chris and Drew had a non-eventful trip back to Syracuse on the 17th. Chris was feeling a bit "off." He was tired and a bit achy when he got back, but put it down to a hectic few weeks. He went into the lab on the 18th, by mid-day he had a throbbing headache. He was also hot to the touch and felt like he was coming down with the flu. He spoke with his advisor who sent him to the Upstate Emergency Room.

7) Why did the advisor send Chris to the E.R.?

In the E.R., Dr. Court took Chris' medical history and vital signs. Chris mentioned to her that he may have been exposed to dengue. Hearing this information, Dr. Court asked for a consult from Dr. Matthew from the Infectious Disease Department. Chris explained to Dr. Matthew that he had just returned from Natal, Brazil, an area that had a high risk of dengue infection. In fact, he had been collecting the species of mosquito, *Ae. aegypti* and *Ae. albopictus,* which are the preferred vectors of the disease.

After confirming Chris' symptoms, Dr. Matthew ordered the following tests to be run: Real Time RT PCR, MAC ELISA, and IgG ELISA. He also filled out the dengue Case Investigation Report. Dr. Matthew explained to Chris that a positive Real Time RT-PCR result would indicate the presence of the virus in his blood. It was not unusual for this particular test to come back negative as the virus is only present for 5 days post infection. The MAC ELISA reports on the presence of the IgM antibody, an antibody that the body makes during the initial phase of an infection which shows up approximately 5 days post fever. A positive MAC ELISA indicates that the patient has recently been exposed to the dengue virus. To be effective the IgG ELISA must be done twice on serum samples collected 14 days apart. If the first sample collected during the early days of infection is negative, but the second sample is positive, then the infection is a primary or first exposure dengue infection. If the first sample is positive and the second sample is positive with a fourfold increase in the IgG titer, it is a secondary exposure to another DENV serotype.

8) If a patient tested positive for a DENV serotype during a Real Time RT PCR test does the patient have dengue? Why or why not?

RUNNING THE TESTS – WHAT WILL THEY REVEAL?

Dr. Matthew drew a blood sample to run the Real Time RT PCR, MAC ELISA, and IgG ELISA tests. He sent the sample over to the lab. Dr. Matthew told Chris that he would have the results back

in a day. In the meantime, he should go home making sure that he drinks fluids and, if needed, take acetaminophen.

Chris went to see Dr. Matthew the next day. Dr. Matthew had received the lab reports back. The Real Time RT PCR was negative; it showed no viral RNA in the blood sample. As the virus is only present in the blood for the first five days after infection, this was not an unexpected result. The MAC ELISA, testing for the presence of IgM, was positive for DENV-2. The IgG ELISA was negative for DENV. Dr. Matthew told Chris that it was likely that he has been infected with DENV-2. The negative IgG ELISA result indicates that this probably is a primary not secondary infection. That determination will be made when you come back in 14 days for another blood draw for the second IgG ELISA.

15 days later – Chris now fully recovered from his symptoms returns to Dr. Matthew's office for the results of his second IgG ELISA test. Dr. Matthew tells Chris that his second IgG ELISA was positive. The official diagnosis is "recent probable primary dengue infection."

9) Explain what Chris' final diagnosis is.

The follow up

Dr. Matthew asked Chris if he remembers being bitten by a mosquito. Chris said he couldn't remember being bitten. About 6 days before they left Natal, he and Drew were sampling in an area of the city that had significantly more pools of stagnant water than the other areas that they had sampled. It seemed as if there were clouds of mosquitoes hovering around some of their sampling spots.

Dr. Matthew asked if Chris would be going back to Brazil to gather more samples in the future; reminding him that an encounter with the DENV-1, 3 or 4 serotypes could lead to dengue hemorrhagic fever and potential death. The body's immune system will produce antigens to the specific DENV serotype it was first exposed to but not to all the serotypes. Dr. Matthew reminded Chris of the symptoms to be aware of: fever lasting 2-7 days during which time the general dengue symptoms will be present. After the fever subsides, he may experience vomiting, abdominal pain and difficulty breathing. This opens a 24-48 hour window where medical attention must be sought as fluids may accumulate in the abdomen and lungs leading to failure of the circulatory system, shock, and death.

This was Chris' last collecting trip in Brazil, he will be now spending his time in the states analyzing his data and writing his dissertation. Citizens of Puerto Rico, U.S. Virgin Islands, Samoa, and Guam are in areas endemic for the virus. While there have been cases of dengue reported in the continental US, these were acquired by travelers when they were outside of the country. Because contact between the *Aedes* mosquito and inhabitants of the continental US is infrequent the probability of a secondary infection is rare. As long as Chris takes precautions when in areas known to harbor dengue, he will not sustain a secondary infection.

10) If you were Chris would you be concerned about a secondary dengue infection? Why or why not?

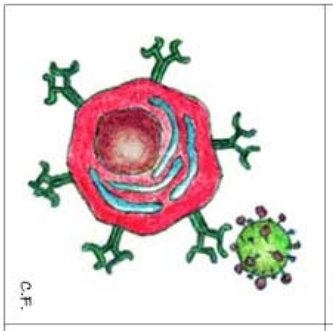
EXTENSION

1) Students can read about the dengue virus on the Protein Data Bank web page. Using Jmol a student can manipulate an envelope protein with antibodies bound and the complete Dengue Virus with antibodies attached.
http://www.rcsb.org/pdb/101/motm.do?momID=103

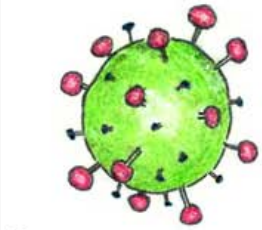
2) Students can construct a paper model of the dengue Virus.
http://www.rcsb.org/pdb/education_discussion/educational_resources/dengue_virus_3Dmodel.pdf

XII. APPENDIX

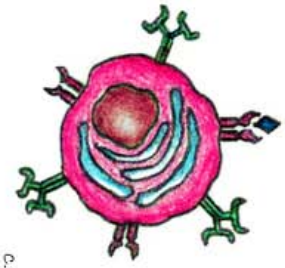
Naïve B Cell



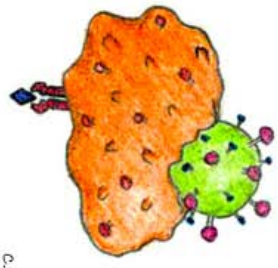
Virus



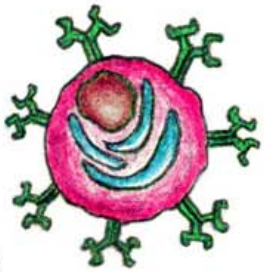
B Cell



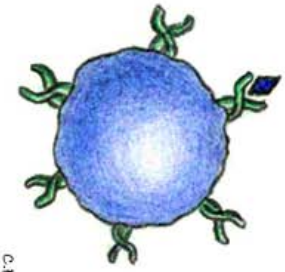
Macrophage engulfing a virus



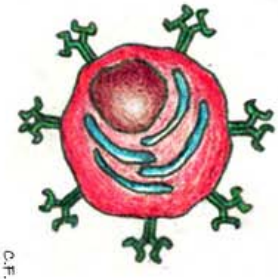
Memory B Cell



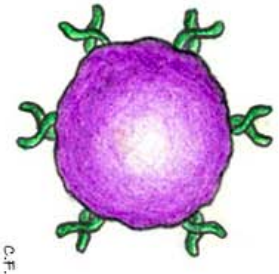
Helper T Cell



Memory B Cell (Duplicate)

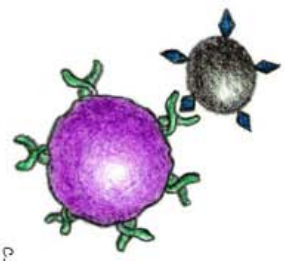


Cytotoxic T Cell



Plasma B Cell with antibodies

Cytotoxic T Cell attacking an infected cell



Humoral (Antibody-Mediated Immunity

Cellular Immunity

What are PCR, RT-PCR and Flow Cytometry?

TEACHERS ANSWERS

I. The following video from the DNA Learning Center at Cold Spring Harbor, elaborates upon the steps that take place in the typical PCR reaction.

https://www.youtube.com/watch?v=2KoLnIwoZKU

1) PCR uses repeated cycles of what to make many copies of DNA?
(Heating and cooling)

2) What causes the DNA to denature into single strands?
(Raising the temperature to near boiling.)

3) What is added to the DNA once the temperature has been decreased and then been raised slightly?
(Short pieces of DNA called primers then Taq Polymerase.)

4) What is the function of the primer and Taq Polymerase?
(The primer locates the DNA section of interest and the Taq Polymerase adds the complementary nucleotides.)

5) How many cycles are needed before the desired DNA sequence starts to accumulate?
(Three)

II. Virtual PCR Lab from Learn.Genetics the Genetics Science Learning Center at University of Utah Health Sciences.

http://learn.genetics.utah.edu/content/labs/pcr/

Define the following vocabulary words located on the home page.

Primer –
(Short DNA pieces that match the segment of DNA to be copied.)

DNA Polymerase –
(Function is to copy DNA. Attaches to a primer and adds the complementary nucleotides.)

Nucleotide –
(The building blocks of DNA, Adenine, Guanine, Thymine and Cytosine.)

Continue on to the PCR Virtual lab and answer the following questions.

1) What is the importance of PCR?
(It can produce billions of copies of a DNA segment of interest.)

2) Where can DNA samples come from for PCR reactions?
(Blood, hair follicles, skin, saliva)

3) To start a PCR reaction what do you need to add to the PCR tube besides the DNA?
(Primer 1, Primer 2, Nucleotides (Adenine, Guanine, Thymine and Cytosine), DNA Polymerase)

4) What is the name of the machine that can heat and cool the PCR tube?
(Thermal Cycler)

5) What happens to DNA at 95^0C?
(It denatures.)

6) Once the DNA Polymerase locates the primers what does it do?
(Add complementary nucleotides to the single stranded DNA.)

7) By the end of cycle three how many copies of the DNA fragment you targeted are there?
(Two)

III. You now have an understanding of the basic principles of PCR. Read the following short article on Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and answer the following questions.
http://www.bio.davidson.edu/people/Kabernd/seminar/2002/method/lowry/RTPCR.htm
1) This technique detects the presence of what nucleic acid?
(mRNA or messenger RNA)

2) If that nucleic acid is present in the sample being tested what does it mean for a specific gene that it is associated with and its product?
(That gene is being expressed and therefore whatever the gene codes for (usually a protein) will be made.)

3) What are the steps in the "RT" portion of RT-PCR?
(Use reverse transcriptase and the appropriate primer to locate the desired mRNA sequence, this will transcribe a complimentary copy of DNA (cDNA), using standard PCR techniques the DNA is replicated using primers and Taq Polymerase.)

IV. What is Flow Cytometry?
Read the information on the Flow Cytometry and answer the accompanying questions.
http://www.biotechnologyforums.com/thread-2185.html
1) What is the function of a flow cytometer?
(To separate out specific cell types.)

2) What is the basis for the separation of different cell types?
(Their size, shape and color that they fluoresce.)

3) Explain two uses for this technique.
(Selecting cells that express a certain gene. Label diseased cells with an antibody to detect the quantity of cells and also to collect the diseased cells for further examination.)

Case Study Answers

1) How is the dengue infection spread?
(Through the bite of female mosquitoes.)

2) How many different strains of dengue are there?
(There are 4 different strains of Dengue.)

3) How is poor water infrastructure contributing to the spread of dengue?
(Mosquitoes need water to lay their eggs in; any stagnant pool of water is a potential breeding ground for the mosquitoes.)

4) If you were Drew, would you be concerned about contracting dengue fever? Support your answer.
(Answers will vary.)

5) What are the symptoms of dengue Fever and dengue Hemorrhagic Fever?
(Dengue fever: high fever; severe headache; pain in the joints, muscle, bone or behind the eyes; rash; mild bleeding. Dengue hemorrhagic fever: fever that lasts from two to seven days inclusive of above symptoms; other symptoms may include vomiting, abdominal pain, breathing difficulties, hemorrhage.)

6) What tests can be used to confirm the disease?
(Real time RT-PCR and MAC ELISA in the acute phase, IgG ELISA in the convalescent phase also to diagnose if the infection is a first exposure or second exposure.)

7) Why did the advisor send Chris to the E.R.?
(Chris had recently been in an area with known dengue disease and he was exhibiting signs of dengue fever.)

8) If a patient tested positive for a DENV serotype during a Real Time RT PCR test does the patient have dengue? Why or Why not?
(Yes, a positive test with Real Time RT-PCR indicates the presence of the dengue protein.)

9) Explain what Chris' final diagnosis.
(This was Chris' first infection with the dengue virus and was with the DENV-2 serotype.)

10) If you were Chris would you be concerned about a secondary dengue infection?
(Answers will vary. If he remains in the continental US, no.)

Detection of the Prevalence of Lyme Disease in Local Canine Populations
Teacher Section

Introduction

What is Lyme disease and what are its symptoms?

Lyme disease is the most common arthropod borne disease in humans. It is highly prevalent in the canine population, particularly in the Northeastern US. The disease is caused by the bacterium *Borrelia burgdorferi*, which resides in the midgut of the deer tick. When a tick bites its host (i.e. takes a blood meal) *B. burgdorferi* moves into the salivary glands of the tick and then, during tick feeding, *B. burgdorferi* moves into its host. The likelihood of infection increases with the duration of tick feeding. It is thought that before 24-48 hours of feeding there is little risk of infection.

What is the Life Cycle of a Deer Tick?

Deer ticks have a life cycle that spans two years, three hosts, and four developmental stages. Eggs are laid in the spring, but the larvae that develop do not carry enough bacteria to cause an infection. Larva in the Northeastern United States feed primarily on the white footed mouse which can harbor the bacteria for long periods before exhibiting symptoms of the disease. The larvae become infected with the bacteria by feeding on the blood of the infected mouse. As winter approaches the larvae leave the mice and enter a resting phase.

The following spring the larvae molt into nymphs. During the spring and summer months the nymphs feed on new hosts, including mice, humans, and dogs. A nymph, during its four day feeding period can be infected by the host it is feeding on, if the host is already infected with *B. burgdorferi*. The nymph, if previously infected in the larval stage, can also infect its host.

During the fall the nymphs molt again, into adult ticks. On average 50% of adult ticks in infected areas in the Northeast US carry the infection. The adult ticks, which can commonly be found on shrubs and other vegetation, will attach to deer and other large animals.

Adult ticks will mate on their new host. Males tend to stay on the host and die whereas females feed for five to seven days then drop off into the leaf debris, where they over winter. The following spring the females will lay eggs, completing the life cycle.

How is Lyme Disease Diagnosed?

An ELISA (enzyme-linked immunosorbent assay) is routinely used to detect the presence of antibodies against *B. burgdorferi* and is generally considered to be evidence for the infection. For a novel infection, an ELISA can detect antibody presence after four to six weeks post infection and as long as 18 months post infection. However, as antibodies are induced by vaccines, the ELISA cannot, in most cases, readily distinguish whether the antibodies were generated following infection or vaccination. However, one commercially available ELISA utilizes an antibody to a peptide that is in the sixth variable region of the VIsE outer membrane protein of *B. burgdorferi* (known as the C_6 peptide). The production of the antibody to this peptide requires that the host was infected by a live organism. Therefore, this ELISA can

distinguish between animals that have had prior exposure from vaccinated or non-infected animals. Another antigen used in ELISAs for the detection of Lyme infection is Decorin binding protein A (DbpA). DbpA is a bacterial outer membrane lipoprotein that facilitates the attachment of *B. burgdorferi* to the tissue of its host. IgG activity is detectable throughout the progression of the disease. In contrast to the C_6 peptide, both vaccinated and infected animals produce DbpA antibodies, and the DbpA ELISA, by itself, cannot distinguish between infected and vaccinated animals.

Canines and Lyme Disease

The studies in this project will identify antibodies (IgG) in the serum of local dogs. Dogs have been chosen for these studies as Lyme disease is one of their most common arthropod borne diseases. Central New York has seen a steady rise in the number of infected dogs over the past several years. This rise has also been mirrored in the human population. Detection of Lyme disease in canines can act as a surrogate detection assay for the risk of human exposure to the disease as both dogs and humans inhabit and exercise in the same areas.

Objectives

Your local veterinary clinic routinely collects blood samples from its canine patients. Utilizing the technique of ELISA, you will determine if canines carry antibodies to the Lyme pathogen in their serum. If so, these studies will demonstrate that the dogs have been exposed to the Lyme pathogen.

Advanced Preparation

How to Make Buffers

1) Phosphate-Buffered Saline (PBS- 1X) 1 Liter

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g

Dissolve the reagents in 800 ml of distilled H_2O and adjust the pH to 7.4 with HCL. Add additional H_2O to make 1 Liter.

2) ELISA Wash Buffer – 1 Liter

TWEEN 20	0.5ml
10% NaN_3	5.0 ml
PBS 1X	994.5 ml

3) Glycine Buffer - 1 Liter

Glycine	7.5g
1.0M $MgCl_2$	1.0 ml
1.0M $ZnCl_2$	0.1 ml

Add distilled H_2O to make 1 Liter.

4) ELISA Block 1 Liter

TWEEN 20	0.5 ml
10% NaN_3	5.0 ml
BSA	2.5 g
PBS 1X	to 1 liter

Materials List

Microtiter Plates
ELISA Wash Buffer
ELISA Blocking Solution
10% Sodium Azide (NaN_3) in milli-q water
Capture antibody - DbpA protein
Canine Serum or Plasma
Goat anti-canine IgG (H+L)-AP
AP Substrate
Plastic wrap
Eppendorf tubes
Pipettors and tips: P1000, P200, P20
Bottles to hold buffers
Sterile pipettes 1ml, 5 ml
Elisa Plate Reader

Materials Price List

Product	Supplier	Order #	Quantity	Price w/o shipping
Phosphatase (AP) Substrate	Sigma Aldrich	S0942-200TAB	1 bottle	$112.00
Tween 20	Sigma Aldrich	P1379-25ml	25 ml	$11.40
Sodium Azide (NaN_3)	Sigma Aldrich	S200205G	5 gm	$12.50
Glycine	Sigma Aldrich	410225-50G	50 gm	$35.10
$MgCl_2$	Sigma Aldrich	M8266-100G	100 gm	$52.10
$ZnCl_2$	Sigma Aldrich	229997-10G	10 gm	$113.50
NaCl	Sigma Aldrich	S7653-250G	250 gm	$35.60
KCl	Sigma Aldrich	P9333-500G	500 gm	$79.90
Na_2HPO_4	Sigma Aldrich	S7907-100G	100 gm	$37.90
KH_2PO_4	Sigma Aldrich	P3786-100G	100 gm	$44.40
Bovine Serum Albumin (BSA)	Sigma Aldrich	05470-5G	5 gm	$261.00
Eppendorf tube 5 ml	Sigma Aldrich	T9661-500EA	500	$50.20
Eppendorf tube 1.5 ml	Sigma Aldrich	T8911-500EA	500	$52.90
Goat anti-canine IgG (H+L)-AP	Southern Biotech	6070-04	1.0 ml	$105.00
DBPA protein	Supplied by Winslow Lab			
Serological Pipettes, Disposable, Sterile, 1.0 ml	Sigma Aldrich	CLS70781N-1000EA	1000	$439.20
Serological Pipettes, Disposable, Sterile, 5.0 ml	Sigma Aldrich	CLS70785N-960EA	960	$651.40
Nuc-Immuno MicroWell 96 well solid plates	Sigma Aldrich	M9410-1CS	Case of 60	$268.00
Pipettors P1000, P200, P20 and associated tips	Donated			
Reagent Bottles	Donated			
ELISA Microplate Reader	Donated			

ELISA Protocol

Prior to starting the experiment, label a mock ELISA plate sheet (see end of protocol).

Day 1 (Coating of the Plate)

1) Coat wells of the microtiter plate with 100ul of a 1μg/100μl capture antibody solution.
2) Wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 2 (Blocking of the Plate)

3) Wash plate with ELISA wash buffer 3 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
4) Block wells by filling each well with 100 μl Blocking Buffer and incubating for 1 hour at room temperature.
5) Wash plate with ELISA wash buffer 2 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
6) Place 100 μl ELISA wash Buffer in wells and wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 3 (Addition of the Primary Antibody – the canine serum/plasma)

7) Flick plate face down into the sink and then tap gently on several paper towels to remove residual fluid.
8) For each different canine serum/plasma sample follow the following dilution scheme

- In well A pipet 196 μl ELISA wash buffer and 4 μl of serum/plasma.
- In wells B-D pipet 100 μl ELISA wash buffer.
- Take 100 μl of liquid from well A and add it to well B, pipet up and down twice to ensure mixing.
- Take 100 μl of liquid from well B and add it to well C, pipet up and down twice to ensure mixing.
- Take 100 μl of liquid from well C and add it to well D, pipet up and down twice to ensure mixing.

Top of Plate	*Dilution*
A	1:50
B	1:100
C	1:200
D	1:400
	Control

9) Cover plate and Incubate at room temperature for 1 hour.
10) Wash plate with ELISA wash buffer 2 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
11) Place 100 μl Blocking Buffer in wells and wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 4 (Addition of the Secondary Antibody)
11) Flick plate face down into the sink and then tap gently on several paper towels to remove residual fluid.
12) To the wells add 100 μl of enzyme coupled AP secondary antibody (goat-anti-canine-IgG-AP) diluted 1:1000 in ELISA Wash Buffer. Incubate at room temperature for 1 hour.
13) Wash plate with ELISA wash buffer 2 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
14) Place 100 μl ELISA Wash Buffer in wells and wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 5 (Addition of the Substrate)
15) Prepare AP substrate; add one pellet of Alkaline Phosphatase to 3 mls of Glycine Buffer. This is enough for approximately 30 wells.
15) Flick plate face down into the sink and then tap gently on several paper towels to remove residual fluid.
16) Add 100 μl of AP substrate to each well.
17) Allow to develop 20 minutes and immediately read on an ELISA plate reader or quench reaction with 50 μl of 3N NaOH per well. If plate is not to be read immediately, cover and place at 4^0C until it is read.
18) Absorbance of the samples is read at 405nm.

Control Protocol
Omit Day 3 steps, no primary antibody is added (serum).

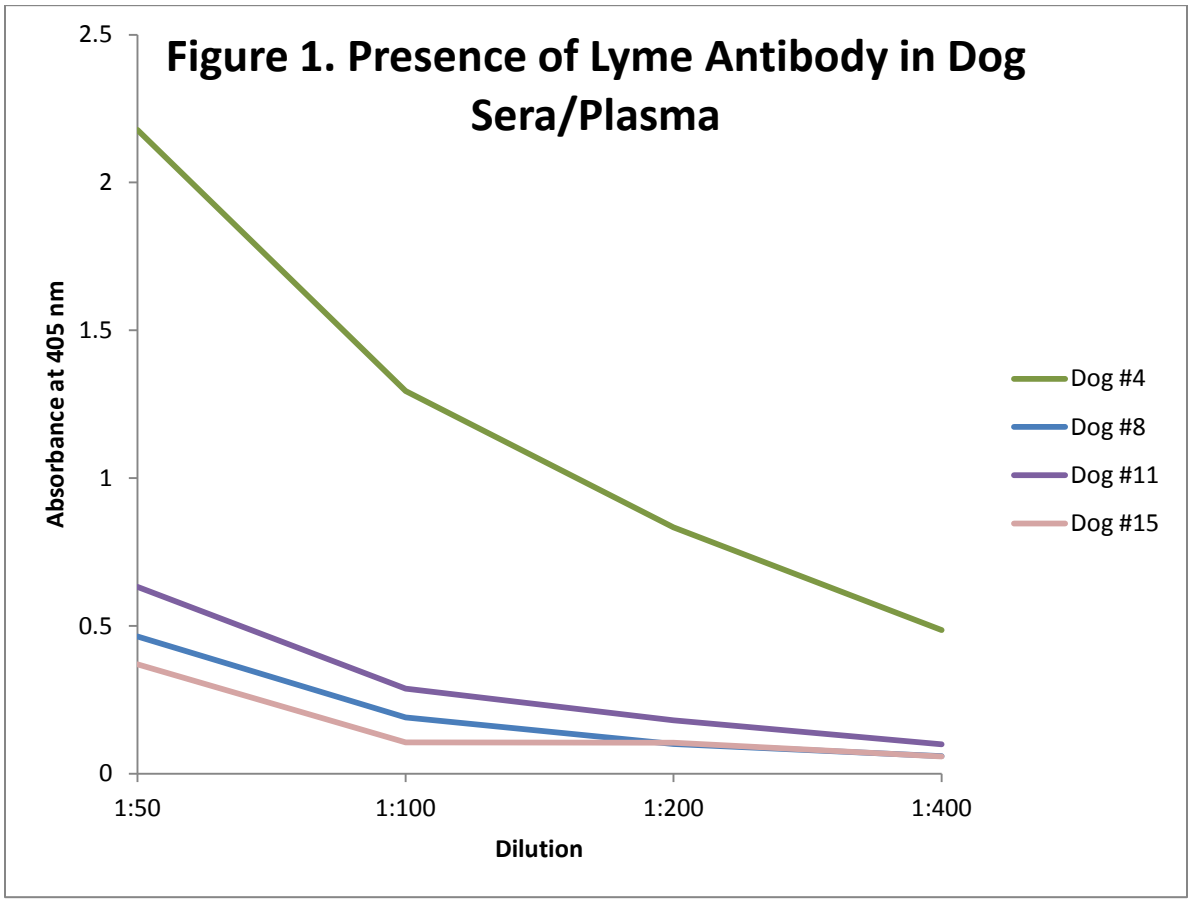
Results
Students will input their numerical results into an excel spread sheet and produce a graph of their data. (See figure 1 for an example.) Original absorbance readings are subtracted from the absorbance reading of the control.

Table 1 Corrected Absorbance Readings at 405 mn of Dog Sera

Dilution	Dog #4	Dog #8	Dog #11	Dog #15
1:50	2.178	0.464	0.632	0.37
1:100	1.295	0.191	0.288	0.106
1:200	0.834	0.101	0.181	0.105
1:400	0.486	0.06	0.1	0.059

= original absorbance – control absorbance
= 0.175 – 0.116
=0.059

Samples that have curves with significantly higher OD values than that of the control will be considered to be positive for Lyme disease.



Format for Laboratory Report

Students will use the mini-poster format. The board of the poster consists of two manila folders that overlap giving the author three surfaces to affix their text. This method is adapted from A Handbook of Biological Investigation. Harrison W. Ambrose III and Katharine Peckham Ambrose. 1995, Hunter Textbooks and Brad Williamson.

Headings:

- Title and Author. The title should be descriptive and include the variables that are manipulated.
- Abstract. The abstract summarizes the experiment including background, results and conclusions. It should be one to two paragraphs in length.
- Introduction. The introduction includes background on the topic and the hypothesis.
- Methodology. This is in paragraph form and includes enough information that a reader familiar with the field could replicate the experiment and data analysis.
- Results. Use graphs, tables and charts to help clarify the data. Include a short summary of what the data mean under the visual used.

- Discussion. Here you discuss what your results mean in relationship to your hypothesis. Point out the significance of your findings. If the results are not what was expected, attempt to explain why, and point out other research avenues.
- Literature Cited. Include all published works mentioned in your report.

References

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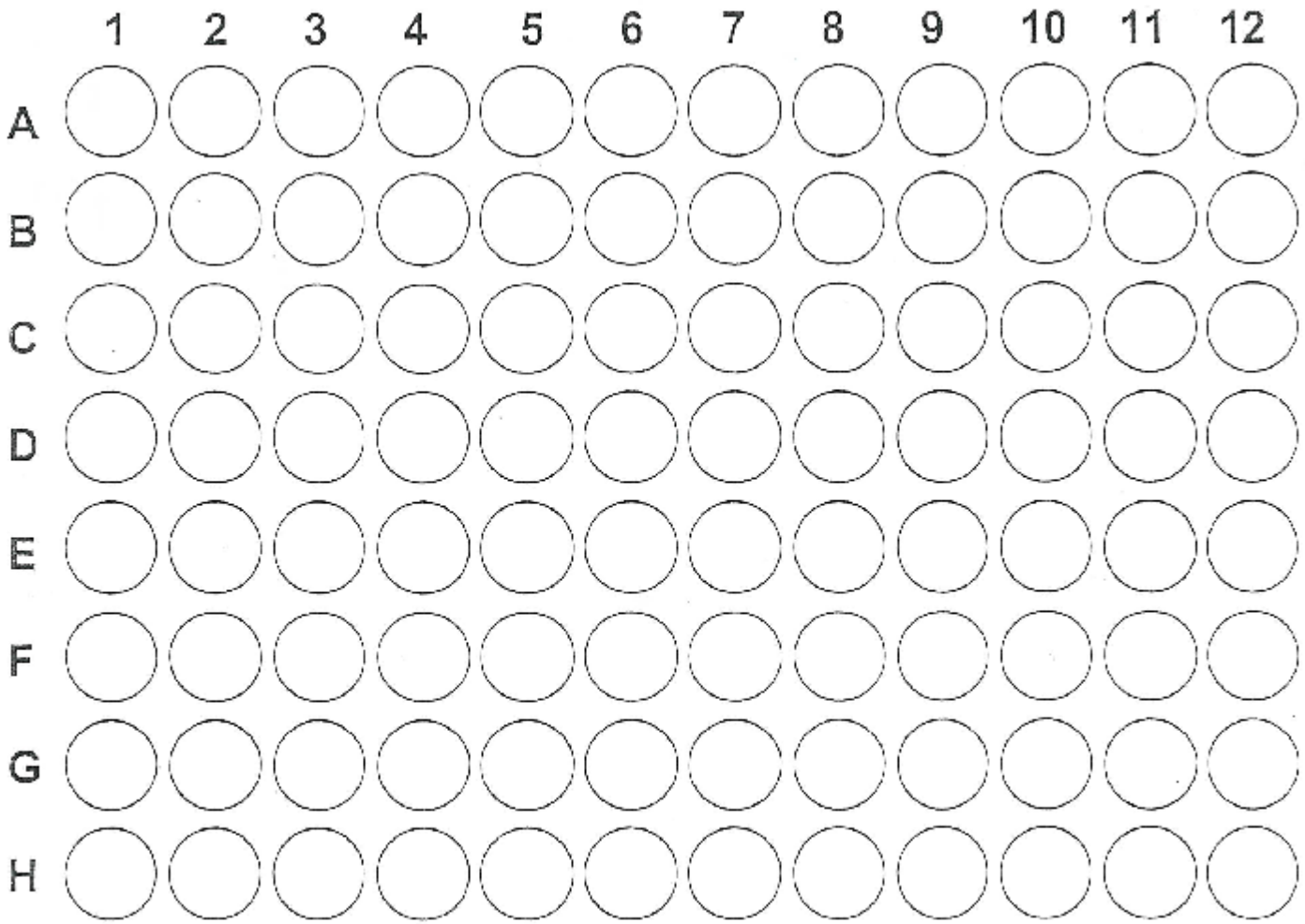
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

Detection of the Prevalence of Lyme Disease in Local Canine Populations
Student Section

Introduction

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What is the Life Cycle of a Deer Tick?

Deer ticks have a life cycle that spans two years, three hosts, and four developmental stages. Eggs are laid in the spring, but the larvae that develop do not carry enough bacteria to cause an infection. Larva in the Northeastern United States feed primarily on the white footed mouse which can harbor the bacteria for long periods before exhibiting symptoms of the disease. The larvae become infected with the bacteria by feeding on the blood of the infected mouse. As winter approaches the larvae leave the mice and enter a resting phase.

The following spring the larvae molt into nymphs. During the spring and summer months the nymphs feed on new hosts, including mice, humans, and dogs. A nymph, during its four day feeding period can be infected by the host it is feeding on, if the host is already infected with *B. burgdorferi*. The nymph, if previously infected in the larval stage, can also infect its host.

During the fall the nymphs molt again, into adult ticks. On average 50% of adult ticks in infected areas in the Northeast US carry the infection. The adult ticks, which can commonly be found on shrubs and other vegetation, will attach to deer and other large animals.

Adult ticks will mate on their new host. Males tend to stay on the host and die whereas females feed for five to seven days then drop off into the leaf debris, where they over winter. The following spring the females will lay eggs, completing the life cycle.

How is Lyme Disease Diagnosed?

An ELISA (enzyme-linked immunosorbent assay) is routinely used to detect the presence of antibodies against *B. burgdorferi* and is generally considered to be evidence for the infection. For a novel infection, an ELISA can detect antibody presence after four to six weeks post infection and as long as 18 months post infection. However, as antibodies are induced by vaccines, the ELISA cannot, in most cases, readily distinguish whether the antibodies were generated following infection or vaccination. However, one commercially available ELISA utilizes an antibody to a peptide that is in the sixth variable region of the VIsE outer membrane protein of *B. burgdorferi* (known as the C_6 peptide). The production of the antibody to this peptide requires that the host was infected by a live organism. Therefore, this ELISA can

distinguish between animals that have had prior exposure from vaccinated or non-infected animals. Another antigen used in ELISAs for the detection of Lyme infection is Decorin binding protein A (DbpA). DbpA is a bacterial outer membrane lipoprotein that facilitates the attachment of *B. burgdorferi* to the tissue of its host. IgG activity is detectable throughout the progression of the disease. In contrast to the C_6 peptide, both vaccinated and infected animals produce DbpA antibodies, and the DbpA ELISA, by itself, cannot distinguish between infected and vaccinated animals.

Canines and Lyme disease

The studies in this project will identify antibodies (IgG) in the serum of local dogs. Dogs have been chosen for these studies as Lyme disease is one of their most common arthropod borne diseases. Central New York has seen a steady rise in the number of infected dogs over the past several years. This rise has also been mirrored in the human population. Detection of Lyme disease in canines can act as a surrogate detection assay for the risk of human exposure to the disease as both dogs and humans inhabit and exercise in the same areas.

Objectives

Your local veterinary clinic routinely collects blood samples from its canine patients. Utilizing the technique of ELISA, you will determine if canines carry antibodies to the Lyme pathogen in their serum. If so, these studies will demonstrate that the dogs have been exposed to the Lyme pathogen.

Advanced Preparation

How to Make Buffers

1) Phosphate-Buffered Saline (PBS- 1X) 1 Liter

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g

Dissolve the reagents in 800 ml of distilled H_2O and adjust the pH to 7.4 with HCL. Add additional H_2O to make 1 Liter.

2) ELISA Wash Buffer – 1 Liter

TWEEN 20	0.5ml
10% NaN_3	5.0 ml
PBS 1X	994.5 ml

3) Glycine Buffer - 1 Liter

Glycine	7.5g
1.0M $MgCl_2$	1.0 ml
1.0M $ZnCl_2$	0.1 ml

Add distilled H_2O to make 1 Liter.

4) ELISA Block 1 Liter

TWEEN 20	0.5 ml
10% NaN_3	5.0 ml
BSA	2.5 g
PBS 1X	to 1 liter

Materials List

Microtiter Plates
ELISA Wash Buffer
ELISA Blocking Solution
10% Sodium Azide (NaN_3) in milli-q water
Capture antibody - DbpA protein
Canine Serum or Plasma
Goat anti-canine IgG (H+L)-AP
AP Substrate
Plastic wrap
Eppendorf tubes
Pipettors and tips: P1000, P200, P20
Bottles to hold buffers
Sterile pipettes 1ml, 5 ml
Elisa Plate Reader

ELISA Protocol

Prior to starting the experiment, label a mock ELISA plate sheet (see end of protocol).

Day 1 (Coating of the Plate)

1) Coat wells of the microtiter plate with 100ul of a 1μg/100μl capture antibody solution.
2) Wrap plate in plastic wrap and incubate overnight at 4^0C.

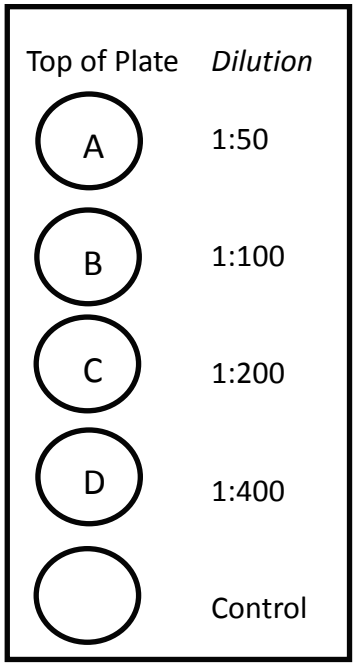
Day 2 (Blocking of the Plate)

3) Wash plate with ELISA wash buffer 3 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
4) Block wells by filling each well with 100 μl Blocking Buffer and incubating for 1 hour at room temperature.
5) Wash plate with ELISA wash buffer 2 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
6) Place 100 μl ELISA wash Buffer in wells and wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 3 (Addition of the Primary Antibody – the canine serum/plasma)

7) Flick plate face down into the sink and then tap gently on several paper towels to remove residual fluid.
8) For each different canine serum/plasma sample follow the following dilution scheme

- In well A pipet 196 μl ELISA wash buffer and 4 μl of serum/plasma and.
- In wells B-D pipet 100 μl ELISA wash buffer.
- Take 100 μl of liquid from well A and add it to well B, pipet up and down twice to ensure mixing.
- Take 100 μl of liquid from well B and add it to well C, pipet up and down twice to ensure mixing.
- Take 100 μl of liquid from well C and add it to well D, pipet up and down twice to ensure mixing.



9) Cover plate and Incubate at room temperature for 1 hour.
10) Wash plate with ELISA wash buffer 2 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
11) Place 100 μl Blocking Buffer in wells and wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 4 (Addition of the Secondary Antibody)
11) Flick plate face down into the sink and then tap gently on several paper towels to remove residual fluid.
12) To the wells add 100 μl of enzyme coupled AP secondary antibody (goat-anti-canine-IgG-AP) diluted 1:1000 in ELISA Wash Buffer. Incubate at room temperature for 1 hour.
13) Wash plate with ELISA wash buffer 2 times by filling each well with 100 μl buffer, flicking plate face down into the sink and then tapping gently on several paper towels to remove residual fluid.
14) Place 100 μl ELISA Wash Buffer in wells and wrap plate in plastic wrap and incubate overnight at 4^0C.

Day 5 (Addition of the Substrate)
15) Prepare AP substrate; add one pellet of Alkaline Phosphatase to 3 mls of Glycine Buffer. This is enough for approximately 30 wells.
15) Flick plate face down into the sink and then tap gently on several paper towels to remove residual fluid.
16) Add 100 μl of AP substrate to each well.

17) Allow to develop 20 minutes and immediately read on an ELISA plate reader or quench reaction with 50 µl of 3N NaOH per well. If plate is not to be read immediately, cover and place at 4^0C until it is read.
18) Absorbance of the samples is read at 405nm.

Control Protocol
Omit Day 3 steps, no primary antibody is added (serum).

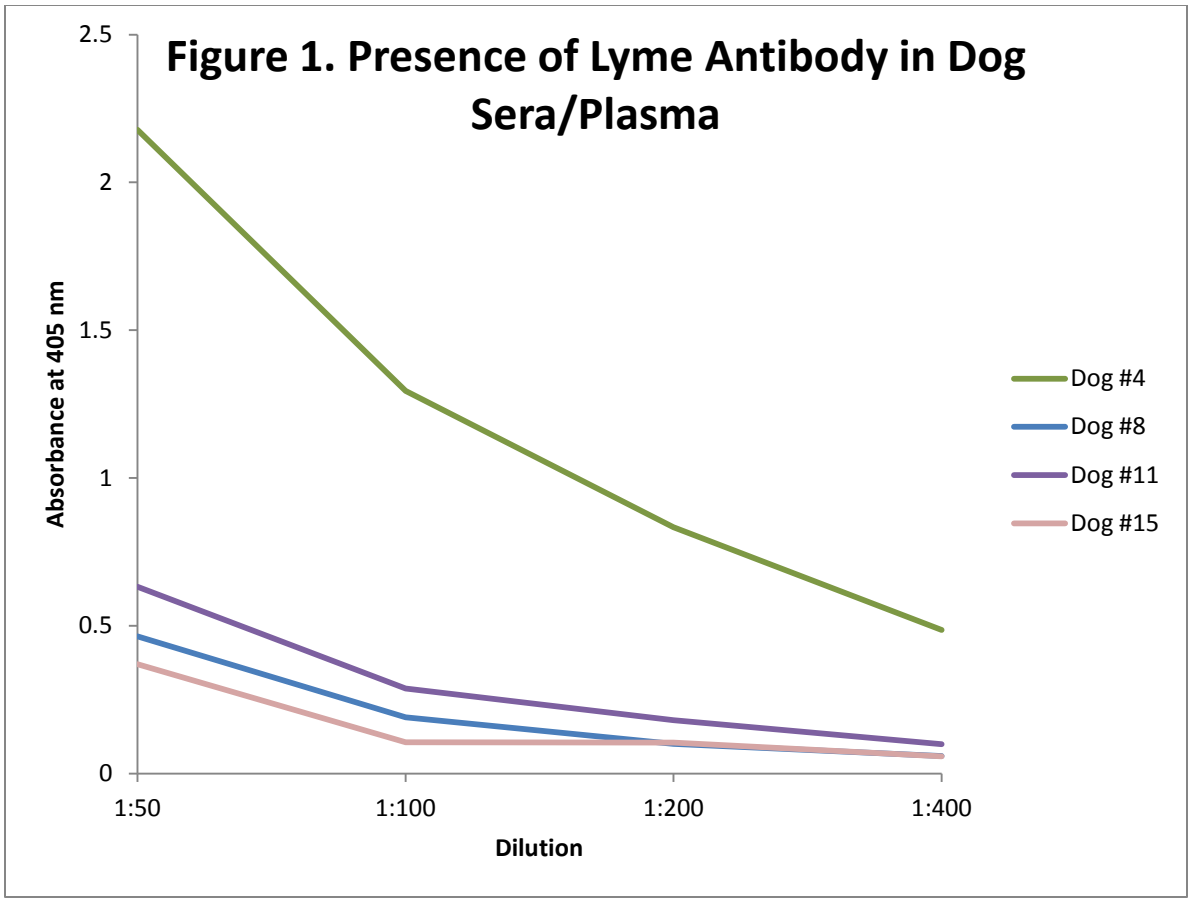
Results
You will input your numerical results into an excel spread sheet. (See Table 1 for an example.) Original absorbance readings are subtracted from the absorbance reading of the control. A graph will be produced of your data. (See Figure 1 for an example.)

Table 1 Corrected Absorbance Readings at 405 nm of Dog Sera

Dilution	Dog #4	Dog #8	Dog #11	Dog #15
1:50	2.178	0.464	0.632	0.37
1:100	1.295	0.191	0.288	0.106
1:200	0.834	0.101	0.181	0.105
1:400	0.486	0.06	0.1	0.059

= original absorbance – control absorbance
= 0.175 – 0.116
=0.059

Samples that have curves with significantly higher OD values than that of the control will be considered to be positive for Lyme disease.



Format for Laboratory Report

You will use the mini-poster format. The board of the poster consists of two manila folders that overlap giving the author three surfaces to affix their text. This method is adapted from A Handbook of Biological Investigation, Harrison W. Ambrose III and Katharine Peckham Ambrose. 1995, Hunter Textbooks and Brad Williamson.

Headings:

- Title and Author. The title should be descriptive and include the variables that are manipulated.
- Abstract. The abstract summarizes the experiment including background, results and conclusions. It should be one to two paragraphs in length
- Introduction. The introduction includes background on the topic and the hypothesis.
- Methodology. This is in paragraph form and includes enough information that a reader familiar with the field could replicate the experiment and data analysis.
- Results. Use graphs, tables and charts to help clarify the data. Include a short summary of what the data mean under the visual used.
- Discussion. Here you discuss what your results mean in relationship to your hypothesis. Point out the significance of your findings. If the results are not what was expected then attempt to explain why and point out other research avenues.

- Literature Cited. Include all published works mentioned in your report.

References

Arnaboldi, P. M., M. Sambir, and R. J. Dattwyler. "Decorin Binding Proteins A and B in the Serodiagnosis of Lyme Disease in North America." Clinical and Vaccine Immunology 21.10 (2014): 1426-436. Web. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4266351/>.

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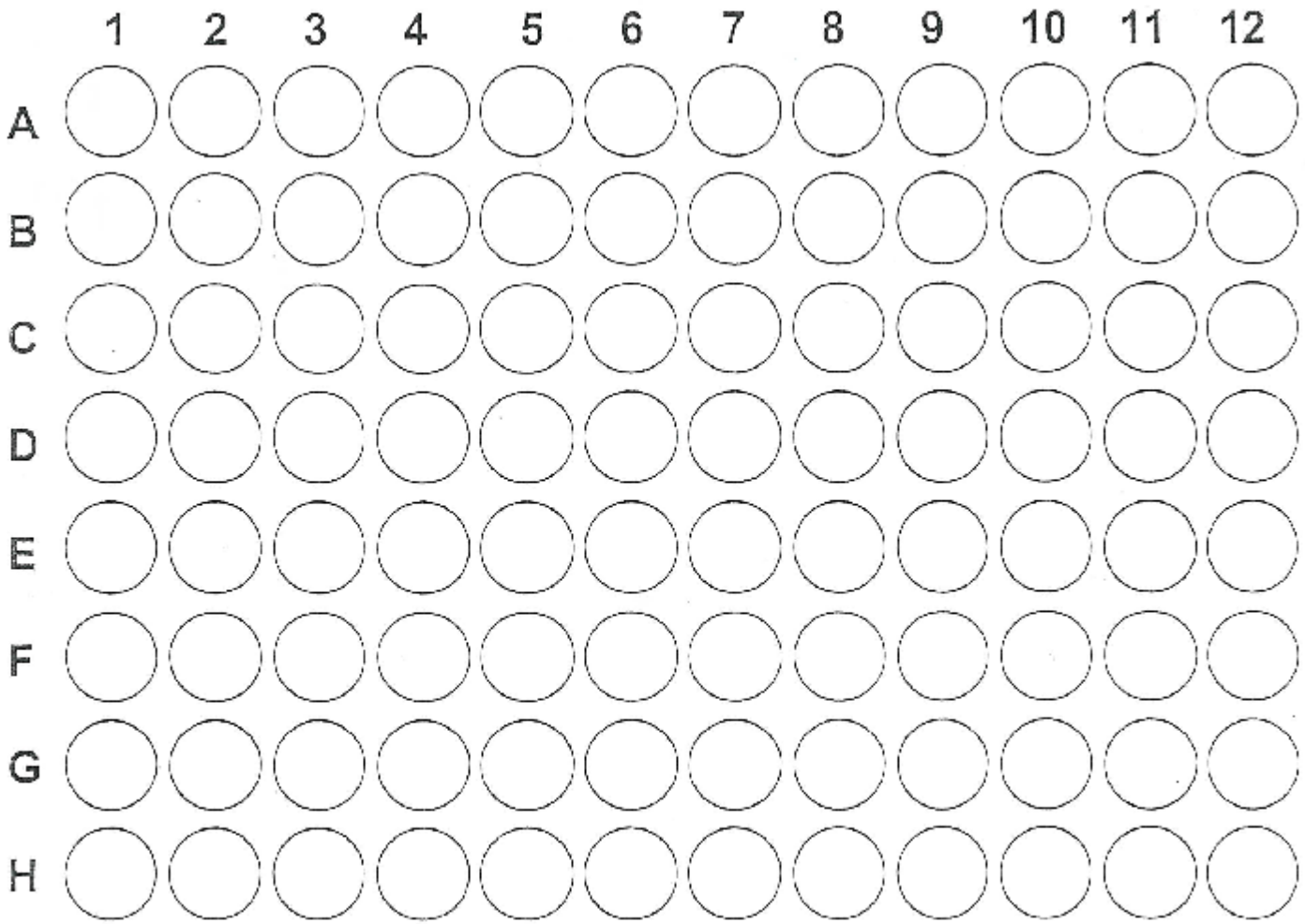
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