Hiding in Plain Site:
The Immune System, Tuberculosis, and Antibiotic Resistance

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HIDING IN PLAIN SITE: THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

TEACHER GUIDE

I. Overview

Hiding in Plain Site: The Immune System, Tuberculosis, and Antibiotic Resistance is a high school biology unit designed to link the study of the immune system with evolution through a closer examination of mycobacterium tuberculosis (mTB). The unit includes teacher presentations and demonstrations as well as internet and laboratory activities.

Students are first introduced to the basic functions of the immune system through its defense against Mycobacterium tuberculosis infection. Then, students work through a simulated ELISA (Enzyme-linked immunosorbent assay) to see its importance in diagnosing disease. Finally, we turn to the emergence of multi-drug resistant mTB as students carry out a laboratory exercise on bacterial transformation. Students present a summary of their learning through the creation of a pamphlet/poster where they investigate a specific disease and describe its symptoms, treatments, suggested causes and immune system interactions. Students will learn about the immune system, the disease Tuberculosis and how bacterial resistance to antibiotics evolves.

This unit may be incorporated into several units of study (biochemistry, disease and immunity, evolution, microbiology, biotechnology) of a typical biology, advanced biology or anatomy and physiology class. It is meant to complement and extend existing curricula rather than exist as a stand-alone unit.

The following Massachusetts Science and Technology/Engineering State Standards are addressed in this unit:

BIOLOGY LEARNING STANDARD 4.7 -- Recognize that communication among cells is required for coordination of body functions. The nerves communicate with electrochemical signals, hormones circulate through the blood, and some cells produce signals to communicate only with nearby cells.

BIOLOGY LEARNING STANDARD 4.8. -- Recognize that the body's systems interact to maintain homeostasis. Describe the basic function of a physiological feedback loop.

BIOLOGY LEARNING STANDARD 5.2 -- Describe species as reproductively distinct groups of organisms. Recognize that species are further classified into a hierarchical taxonomic system (kingdom, phylum, class, order, family, genus, species) based on morphological, behavioral, and molecular similarities. Describe the role that geographic isolation can play in speciation.

BIOLOGY LEARNING STANDARD 5.3 -- Explain how evolution through natural selection can result in changes in biodiversity through the increase or decrease of genetic diversity within a population.

II. Science background:

This is a guided unit for students to discover the functions of the human immune system, information on tuberculosis, a world-wide public health threat, and a link between the two and evolutionary theory. Prior to beginning this unit teachers should introduce students to the overall, big-picture functioning of the immune system (the first, second, and third lines of defense, the inflammatory response, phagocytosis, antibody-mediated and cell-mediated immunity, and all of the cell "players" (i.e. helper-T cells, B cells, Dendritic cells, etc) and provide students with information on basic tuberculosis facts (what is tuberculosis, how it is spread, latent TB infection and TB disease, and world-wide incidence). Students

should also be familiar with the concepts of evolutionary theory. Teachers are encouraged to utilize the brief online video from PBS's Teacher's Domain, *Evolving Ideas: Why Does Evolution Matter Now?* as a lead in to this unit. This video will introduce multi-drug resistant tuberculosis and highlight one reason why it is important to understand evolution. (http://www.teachersdomain.org/)

Another good resource for immune system information comes from the Harvard University Life Sciences/HHMI Outreach Program. Here you can find a number of online lectures and animations for the immune system. http://outreach.mcb.harvard.edu/index.htm Look for video "Understanding Resistance to Tuberculosis" by Dr. Barry Bloom at http://outreach.mcb.harvard.edu/videos.htm

III. Student Outcomes:

The content of this unit covers the immune system, tuberculosis, and antibiotic resistance. It allows students to investigate and experience the processes of antibody-antigen reactions in an ELISA assay and perform a bacterial transformation in a way to relate the immune system and the problem of antibiotic resistance.

IV. Learning Objectives:

1. Students will be able to list and describe the two main defensive strategies of the immune system: innate immunity and acquired immunity.
2. Students will be able to distinguish between antibody-mediated immunity and cell-mediated immunity.
3. Students will be able to compare and contrast the cell components of the immune system.
4. Students will be able to distinguish between antigens and antibodies.
5. Students will be able to list and draw the different classes of immunoglobulins.
6. Students will be able to describe the basics of an ELISA assay and practical uses of this type of lab assay.
7. Students will research and describe the disease tuberculosis and how it interacts with the human immune system.
8. Students will perform a bacterial transformation.
9. Students will relate the bacterial transformation lab to evolution in general and to the evolution of bacterial resistance in particular.
10. Students will communicate their knowledge of the immune system with two narrative essays.

V. Time Requirements:

Total time for this unit is approximately 7 class periods (7 hours – although the computer exercises may be completed by students at home or at the library). The immune system web tutorial is 1 class period, the antibody investigation is 1 class period, the virtual ELISA is 1 class period, the ELISA Classroom Simulation is 1 class period, the bacterial transformation lab requires 3 – 60 minute lab periods.

VI. Advanced Preparation:

Teachers should make sure students have access to a computer lab if the students are to conduct the lab simulations during the school day.

The ELISA: HIV/AIDS Test Simulation Lab (526706WW, $89.95) can be ordered from Frey Scientific (http://store.schoolspecialtyonline.net/)

The bacterial transformation lab (595903WW, $109.95) can be ordered from Frey Scientific (http://store.schoolspecialtyonline.net/)

VII. Materials and Equipment:

- Computer with Internet Access
- For ELISA – most materials are found in the kit.

- For Bacterial Transformation – most materials are found in the kit. Items not found in the kit include:
    - Marker
    - Clear tape
    - Microfuge tube rack
    - Container with ice
    - Hot plate with large beaker of water at 42oC
    - Incubator (37oC)
    - Microwave

VIII. Student Prior Knowledge and Skills:

Prior to completing the culminating narrative essays of this unit connecting the immune system, tuberculosis and the evolution of antibiotic resistance, the students will gain knowledge of the immune system, tuberculosis and antibiotic resistance through a variety of lectures, written assignments, computer lab simulations and wet labs. Students should also have a solid background in basic experimental techniques. Students should be familiar with lab safety and measurement procedures.

IX. What is Expected from Students:

Students are expected to learn how the immune system coordinates its resources to fend off attacks to the body. They will also learn about the disease tuberculosis and how the causative agent Mycobacterium tuberculosis is evolving resistance to many of the antibiotics currently used to treat it. They will learn the mechanism of bacterial transformation as well as the basics of the ELISA assay and be able to explain the reasons behind the procedures.

X. Anticipated Results:

Answers to the lab, discussion and activity questions may be found in the appendix

XI. Classroom Discussion:

Answers to the lab questions may be found in the appendix

XII. Assessment:

Students may be assessed on their ability to master the learning objectives listed above, their completion of all activities listed under materials in the student section below, their understanding of the biological principles applied to both the ELISA test and the bacterial transformation laboratory (via discussion questions listed with each activity) and in discussions of their results

XIII. References:

- http://bcs.whfreeman.com/thelifewire8e/ [viewed 2/13/2010]
- http://www.hhmi.org/biointeractive/vlabs/immunology/index.html [viewed 2/13/2010]
- http://dujs.dartmouth.edu/winter-2009/new-trickes-for-an-old-foe-the-threat-of-antibiotic-resistant-tuberculosis[viewed 2/13/2010]
- http://www.phschool.com/science/biology_place/labbench/ [viewed 2/13/2010]
- http://drake.marin.k12.ca.us/academics/rock/ROCK_Documents.htm [viewed 2/13/2010]
- http://store.schoolspecialtyonline.net/ [viewed 3/8/2010]
- http://outreach.mcb.harvard.edu/index.htm [viewed 2/13/2010]
- http://outreach.mcb.harvard.edu/videos.htm [viewed 2/13/2010]

STUDENT SECTION

I. Rationale

As you are reading this laboratory guide your body is under attack! Pathogens – viruses, bacteria, protists and fungi – are trying to penetrate your protective outer coverings in an attempt to get a foothold and carry out their own life activities. When you think about it, your body is an ideal place for pathogens to grow and reproduce as there is a rich supply of nutrients within a protected setting. And these pathogens are everywhere, on every surface, in every environment. What's keeping them at bay is your immune system.

II. Materials

a. **Computer with Internet Access**
b. **Student worksheets:**
    i. **Hiding in Plain Site - Student Lab Guide – Introduction.docx**
    ii. **Hiding in Plain Site - Student Lab Guide – Part 1.docx**
    iii. **Hiding in Plain Site - Student Lab Guide – Part 2.docx**
    iv. **Hiding in Plain Site - Student Lab Guide – Part 3.docx**
    v. **Hiding in Plain Site - Student Lab Guide – Part 4.docx**
        1. **Bacterial Transformation Lab Protocol**
    vi. **Hiding in Plain Site - Student Lab Guide – Part 5.docx**

III. Procedures

YOUR ASSIGNMENT:
In the first part of this 5 part activity you will explore how a normal immune system functions to spare you the fate of an infection. You will learn the roles of the cells and organs of the body that make up the immune system. In later activities you will gather information concerning the actions of the immune system in fighting a specific battle against one type of pathogen – bacteria, specifically a battle against *Mycobacterium tuberculosis* – the bacterium that causes the disease tuberculosis. Through both a web tutorial and a simulated laboratory exercise you will learn how antibodies assist with immunity and with research and medicine. Then, you will investigate how bacteria mutate and gain resistance to the antibiotics used to treat people with bacterial infection. Finally, you will communicate your results by discussing what you have learned about the immune system, tuberculosis, and antibiotic resistance.

THE PLAN OF ACTION:

- Part 1 - Carry out the Immune System Web Tutorial
- Part 2 - Investigate Antibodies – Workhorses of Immunity and Research
- Part 3 - Carry out the Tuberculosis Web Investigation/Fact Sheet
- Part 4 - Conduct the Bacterial Transformation Lab
- Part 5 - Communicate your Results

To implement this plan please begin with **PART 1: IMMUNE SYSTEM WEB TUTORIAL**

IV. Data Collection

Collect your data on the worksheets provided. For the ELISA: HIV/AIDS Test Simulation lab as well as the Bacterial Transformation activity please use the procedures provided by your teacher.

V. Discussion/Analysis

Throughout this unit you have learned about the immune system, how the principle of antibody/antigen interaction is used to indirectly test for the presence or absence of disease causing pathogens such as HIV or Mycobacterium tuberculosis, tuberculosis disease and how bacteria evolve through transformation. As you have no doubt learned during the Tuberculosis Web Investigation/Fact Sheet lesson, MTB is the scourge that it is upon humanity because of its unique growth and anatomical characteristics. It has a cell wall that is particularly good at protecting the cell and the bacterium has a very slow cell division rate. This slow reproduction rate along with human error during the long treatment process allows the microbe sufficient time to mutate via evolution-based methods.

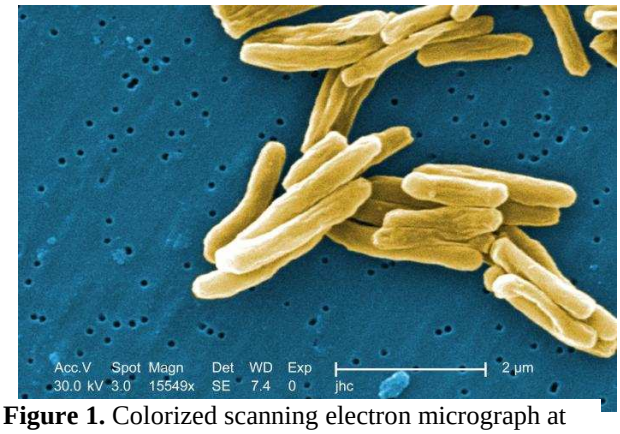
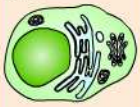
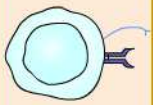
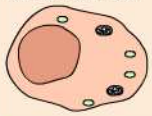
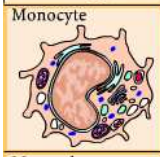
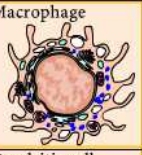
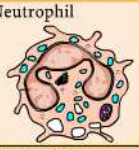
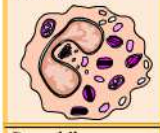
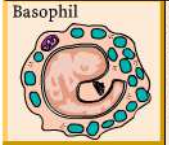


Figure 1. Colorized scanning electron micrograph at 15549x magnification, showing details of the cell wall configuration of tuberculosis bacteria. The cell wall is a key part of the pathogen.

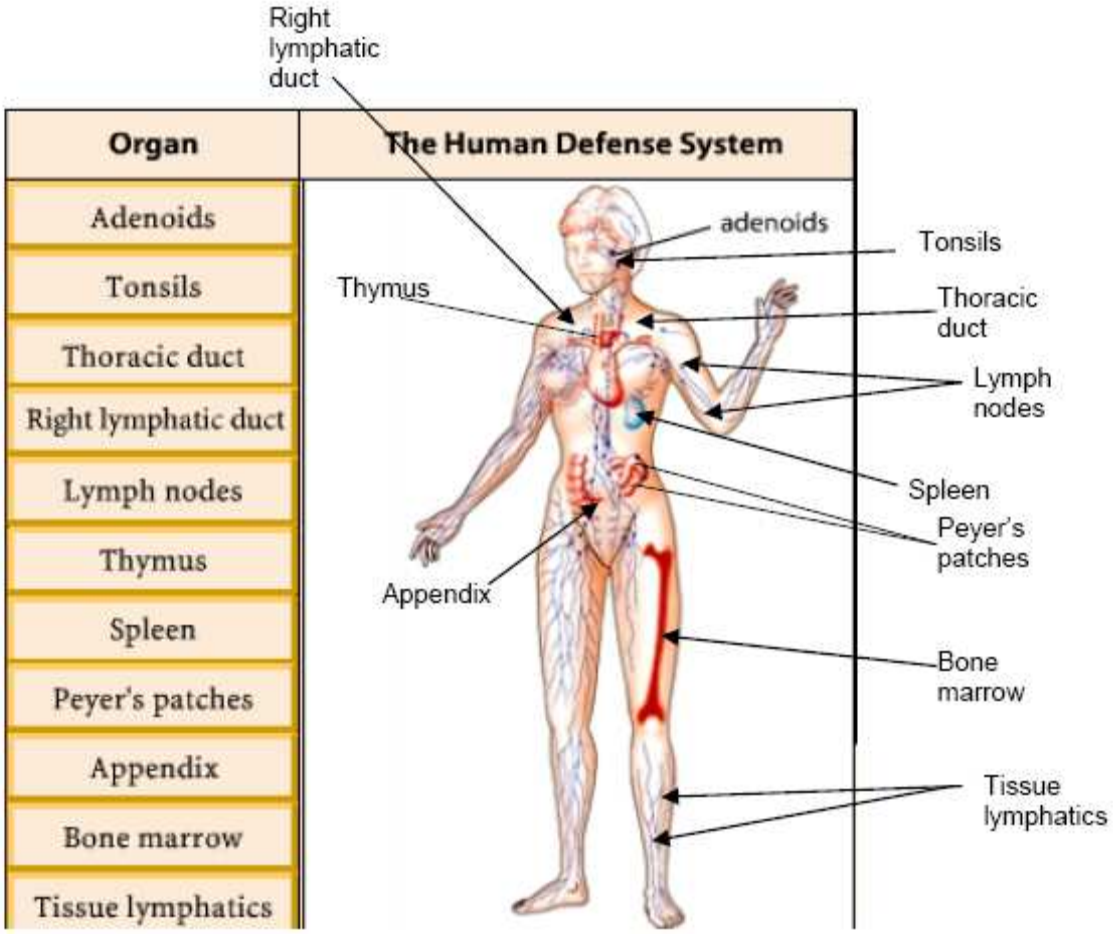
In Part 5 - Communicate your Results section of this activity you are to describe, in two narrative essays, how a tuberculosis infection affects a person and from the microbes point of view, how it evades the immune system.

VI. Appendix – Suggested Answers

Part 1, Page 4 Table, Cells of the Immune System

Cell Type	Function
B cell 	A B cell differentiates to form plasma cells, which secrete antibodies; and memory cells, which provide immunity to future infections by a familiar pathogen. B cells make up 10-20% of lymphocytes. Lymphocytes make up 15-50% of white blood cells. PLAY
T cell 	T helper cells (also called T_H or $CD4^+$ cells) assist both the cellular and humoral immune systems by secreting cytokines. Cytotoxic T cells (also called T_C or $CD8^+$ cells) recognize and kill virus-infected cells and other altered-self cells. T cells make up 68-75% of lymphocytes. PLAY
Natural killer cell 	Natural killer cells attack and lyse virus-infected or cancerous cells of the body. Natural killer cells make up 5–10% of lymphocytes in the blood. PLAY
Cell Type	**Function**
Monocyte 	Monocytes circulate in the bloodstream, migrate into other tissues, and differentiate into macrophages. Monocytes make up 1–6% of white blood cells. PLAY
Macrophage 	Macrophages are located in a variety of the body's tissues. They are phagocytic cells that engulf and digest microorganisms. They also activate T cells by releasing cytokines. PLAY
Dendritic cell 	Dendritic cells are located in a variety of the body's tissues. They are potent antigen-presenting cells that present antigens to T cells. PLAY
Cell Type	**Function**
Neutrophil 	Neutrophils respond rapidly to inflammation, moving from the blood into the inflamed tissue, where they phagocytize debris and pathogens. Neutrophils also phagocytize antibody-coated pathogens. Neutrophils make up 50–70% of white blood cells. PLAY
Eosinophil 	Eosinophils migrate from the blood to other tissues and kill antibody-coated parasites. Eosinophils make up 1–3% of white blood cells. PLAY
Basophil 	Along with MAST CELLS, basophils release histamine, which plays a major role in inflammation and an allergic response. Basophils also may promote the development of T cells. Basophils make up <1% of white blood cells. PLAY

Part 1, Page 5 Organs of the immune system



Part 1, Page 5 Questions 1 and 2

1. One milliliter of blood typically contains 7 million WBC and 5 billion RBC.

2. The lymph nodes as well as the spleen contain large numbers of B cells, T cells and macrophages. In these organs the lymphocytes can efficiently recognize pathogens, interact with each other and become activated to defend the body.

Part 1, Page 6 Questions 3-7

3. They are displayed on the surfaces of the macrophages
4. Helper T cells become activated by binding to the antigens on the macrophage surface. Interleukin 1 is a cytokine released by the macrophage upon binding to activate the Helper T cell.
5. The Helper t cell releases its own cytokines to stimulate the Helper T cell to proliferate.
6. The B cell is activated once it binds to a T Helper cell. The T Helper cell releases cytokines to stimulate the B cell to proliferate. The resulting B cells are either long lasting memory B cells or antibody secreting plasma cells.
7. Plasma cells have numerous ribosomes and extensive endoplasmic reticulum membranes.

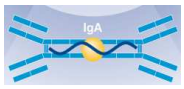
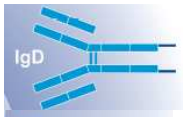
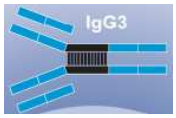
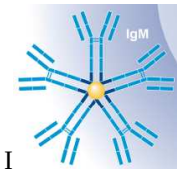
Part 1, Page 7 Questions 8-11

8. The cytotoxic T cell has T-cell receptors that are specific for the displayed antigen on the infected cell.
9. The cytotoxic T cell binds to the infected cell displayed antigen and becomes activated.
10. Once activated the cytotoxic T cell proliferates. The cytotoxic T cells all have the same specificity to the antigenic determinant. Once it encounters the same antigen on another infected cell, the cytotoxic T cell releases perforin molecules which poke holes in the target cell causing it to lyse and die.
11. All body cells display proteins on their surface. Early in their development cytotoxic T cell as well as Helper T cells undergo a screening test to make sure they do not recognize "self" cell proteins. Foreign cells have 'nonself' proteins and thus are recognized and attacked. Cancerous cells produce mutant proteins that are also recognized as 'nonself' and are attacked.

Part 2, Page 9 Questions 1-3

1. There are 5 classes of immunoglobulins in mammals
2. IgA immunoglobulin is the most produced antibody in mammals.
3. IgM appears in all vertebrates

Part 2, Page 10 Table1
Immunoglobulin classes found in mammals

Immunoglubulin	**Drawing**		**Major**	Characteristics
IgA		150 to 600	Monomer to tetramer	Most produced Ig. Found in mucosal areas, such as the gut, respiratory and urogenital tract, and prevents their colonization by pathogens. Resistant to digestion and is secreted in milk.
IgD		150	Monomer	Function unclear. Works with IgM in B-cell development; mostly B cell bound
IgE		190	Monomer	Binds to allergens and triggers histamine release from mast cells and is involved in allergy. Also protects against parasitic worms.
IgG		150	Monomer	Major Ig in serum. Provides the majority of antibody based immunity against invading pathogens. Moderate complement fixer (IgG3) can cross placenta.
IgM	 I	900	Pentamer	First response antibody. Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated immunity before there is sufficient IgG.

4. Systemic Lupus Erythematosus
5. Sera/Serum is a clear watery fluid obtained after removing blood cells and other components from blood by centrifugation that will contain antibodies. The purple tube was added as a counterbalance in the centrifuge.
6. Serial dilutions are made in order to determine the level of the antibody in the sample. Highly diluted samples will not appear positive if there is a low titer of antibody in the sera.
7. A plastic 96-well ELISA Microplates, Flat-bottom
8. Proteins such as antigens and other biological materials can, under proper conditions, physically bind to the plastic material composing the wells of the ELISA plate.

   The coating procedure must be done carefully. If too little antigen is used, bare spots will permit antibody or other protein to stick, leading to a false-positive reaction. If too much antigen is used, the excess will be able to bind SLE antibody from patient sera but then will be washed away, creating a false-negative reaction.

   The addition of antigen is the crucial first step in the chain of recognition events between antigen and antibody that will end with the formation of color from the enzyme bound to the second antibody.
9. An ELISA may be subject to many errors. One is that the biological and chemical reagents used in ELISA can change with time. Another is that the ELISA is not always conducted under appropriate conditions. To rule out such problems, two controls are used. One control should always produce a positive response if the reagents and conditions are correct. The second control should never produce a positive response. If either control sample fails to react as expected, then the results for the patients' samples cannot be trusted and the assay must be repeated.
10. Washing helps remove any antibody that did not react with the SLE antigen in the well. When the fluid is removed from the well, antibody that has reacted with antigen remains attached to the well surface. Unreacted (unbound) antibody may also remain in the well in the small amount of fluid that is left behind. This unbound antibody must be removed, because the anti-human antibody added in the next step will recognize and react with any antibody remaining in the well, regardless of whether that antibody is specific for the SLE antigen. A reaction with non-SLE antibody will produce a false-positive result.
11. The second antibody, unlike the first, does not recognize the SLE antigen. Instead, rabbit anti-human antibody reacts with human antibody. SLE antibody is a human antibody that may be present in a well because it is being held by antigen. The second antibody (from rabbit) will therefore recognize this antibody and bind to it. If the well has not been washed thoroughly, other human antibody may still be there and will also react with the second antibody. Reaction of a non-SLE human antibody with the second antibody will produce a false-positive result.
12. HRP- horseradish peroxidase is an enzyme used to stimulate the conversion of the colorless substrate into a colored product in this exercise. In this example, HRP (the enzyme) will interact with a substrate called ABTS (2,2'-azinobis-3-ethylbenzothiazoleine-6-sulfonic acid) to produce a yellow solution.
13. Summarize the results of the ELISA plate. Which patient is likely to have SLE? This shows that patient A is likely to have SLE

A	B	C	+	-
x	x		x	
x	x		x	
x			x	

14. ELISA Protocol summary
    a. The sample is bound to the ELISA plate
    b. A primary antibody is added to the plate. It binds with the sample antigen
    c. A secondary antibody (detection) with enzyme is added. The secondary enzyme-linked antibody binds to the primary antibody. If there are no primary antibody's (meaning the sample did not contain the appropriate antigen), then the secondary detection antibody would not bind
    d. A substrate is added. Those wells with the appropriate antigen and thus with the primary antibody would react with the detections antibody enzyme giving a colored product.

Part 3, Pages 13-14 Tuberculosis Web Investigation – Fact Sheet

i. Suggested Answers to Basic Information Fact Sheet Tuberculosis
    a. Robert Koch in 1882
    b. TB is caused by the bacterium *Mycobacterium tuberculosis*
    c. No. The human version of TB is only found in humans. It does not have a natural source such as found in the soil, etc. It is only transmitted form human to human.
    d. TB is a disease of the poor. It is found around the world, primarily in areas where there is a lack of proper medicine.
    e. Overall, one-third of the world's population is currently infected with the TB bacillus.
    f. Anyone can get TB, although the risk is higher for people living in a part of the world where TB is prevalent.
    g. TB disease can be either chronic or acute. Left untreated, each person with active TB disease will infect on average between 10 and 15 people every year. But people infected with TB bacilli will not necessarily become sick with the disease. The immune system "walls off" the TB bacilli which, protected by a thick waxy coat, can lie dormant for years. When someone's immune system is weakened, the chances of becoming sick are greater.
    h. Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel TB germs, known as bacilli, into the air. A person needs only to inhale a small number of these to be infected.
    i. Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist: latent TB infection and active TB disease. TB bacteria can live in your body without making you sick. This is called **latent TB infection (LTBI)**. In most people who breathe in TB bacteria and become infected, the body is able to fight the bacteria to stop them from growing. People with latent TB infection do not feel sick and do not have any symptoms. The only sign of TB infection is a positive reaction to the tuberculin skin test or special TB blood test. People with latent TB infection are not infectious and cannot spread TB bacteria to others. However, if TB bacteria become active in the body and multiply, the person will get sick with TB disease. TB bacteria become active if the immune system can't stop them from growing. When TB bacteria are active (multiplying in your body), this is called **TB disease**. TB disease will make you sick. People with TB disease may spread the bacteria to people they spend time with every day. Many people who have latent TB infection never develop TB disease. Some people develop TB disease soon after becoming infected (within weeks) before their immune system can fight the TB bacteria. Other people may get sick years later, when their immune system becomes weak for another reason.
    j. When the inhaled tuberculosis bacteria enter the lungs, they can multiply and cause a local lung infection (pneumonia). The local lymph nodes associated with the lungs

may also become involved with the infection and usually become enlarged. The hilar lymph nodes (the lymph nodes adjacent to the heart in the central part of the chest) are often involved. In addition, TB can spread to other parts of the body. The body's immune (defense) system, however, can fight off the infection and stop the bacteria from spreading. The immune system does so ultimately by forming scar tissue around the TB bacteria and isolating it from the rest of the body. Tuberculosis that occurs after initial exposure to the bacteria is often referred to as primary TB. If the body is able to form scar tissue (fibrosis) around the TB bacteria, then the infection is contained in an inactive state. Such an individual typically has no symptoms and cannot spread TB to other people. The scar tissue and lymph nodes may eventually harden, like stone, due to the process of calcification of the scars (deposition of calcium from the bloodstream in the scar tissue). These scars often appear on x-rays and imaging studies like round marbles and are referred to as a granuloma. If these scars do not show any evidence of calcium on x-ray, they can be difficult to distinguish from cancer. Sometimes, however, the body's immune system becomes weakened, and the TB bacteria break through the scar tissue and can cause active disease, referred to as reactivation tuberculosis or secondary TB. For example, the immune system can be weakened by old age, the development of another infection or a cancer, or certain medications such as cortisone, anticancer drugs, or certain medications used to treatarthritis or inflammatory bowel disease. The breakthrough of bacteria can result in a recurrence of the pneumonia and a spread of TB to other locations in the body. The kidneys, bone, and lining of the brain and spinal cord (meninges) are the most common sites affected by the spread of TB beyond the lungs.

k. TB and history: Partial Listing from Wikipedia. http://en.wikipedia.org/wiki/Tuberculosis_in_popular_culture

Through its affecting important historical figures, **tuberculosis** has influenced particularly European history, and become a theme **in art** – mostly literature, music, and film.

Opera and theatre:

- Mimì, the heroine of Puccini's opera, *La bohème* has tuberculosis.
- Marguerite Gautier, heroine of Alexandre Dumas, fils' novel and play *The Lady of the Camellias*, dies of tuberculosis. The same story was adapted as the opera *La Traviata* by Giuseppe Verdi (heroine's name changed to Violetta Valéry) and, more loosely, as the movie *Moulin Rouge!* where Satine dies of tuberculosis.
- Edmund, the protagonist of Eugene O'Neill's *Long Day's Journey into Night* is diagnosed with TB at the start of the play, which deals, in part, with his subsequent mental anguish.
- The character of Jody in the play Hollywood Arms (by Carrie Hamilton and Carol Burnett) has TB.
- The play *The Cripple of Inishmaan* has themes of TB involving the protagonist and another character.

- "Chopin and The Nightingale": a dramatic reading with music in six acts for narrator, two sopranos and piano. It enacts the true-life romance of Chopin and Jenny Lind with reference to The Nightingale story by Hans Christian Andersen. Playwrights: Cecilia and Jens Jorgensen,Icons of Europe (Brussels).

Novels:

- The latter half of Erich Maria Remarque's novel Three Comrades focuses on Patricia Hollman's love of life in light of her ultimately futile struggle with tuberculosis.
- Tuberculosis patients were frequent characters in 19th century Russian literature, examples of which include Katerina Ivanovna from Fyodor Dostoevsky's Crime and Punishment, Kirillov from Dostoevsky's Demons (aka The Possessed), and Ippolit and Marie from Dostoevsky's The Idiot.
- Thomas Mann's The Magic Mountain takes place at a Sanitarium where all the characters suffer from tuberculosis.
- In the novel The Constant Gardener by John le Carré, as well as in the movie adaptation directed by Fernando Meirelles, the plot largely revolves around TB drugs beings tested on unwitting subjects in Africa, and dire predictions about a global pandemic of a drug-resistant form of the disease appear repeatedly.
- Richard Yates, (1926-1992), the American writer, suffered from TB shortly after WWII, and wrote about the disease in a number of his short stories, including "No Pain Whatsoever"
- Fantine in Victor Hugo's novel Les Miserables becomes ill and ultimately dies from "consumption".
- Smike in Charles Dickens' novel "Nicholas Nickleby" dies from the "dread disease"
- Sheilagh Fielding in Wayne Johnston's The Colony of Unrequited Dreams has tuberculosis, despite her father being a doctor, which brings shame upon her family in Newfoundland.
- Upton Sinclair's novel The Jungle portrays tuberculosis as common among bovine in the meat-packing plants of Chicago; consumption is a common illness for packers.
- Marie-Claire Blais's Une Saison dans la vie d'Emmanuel (A Season in the Life of Emmanuel), 1965, features a teenaged main character, Jean-le-Maigre ("Skinny John") whose exploration of his gay sexual orientation in traditional rural Quebec is cut short by his death from tuberculosis.
- In Barbara Hambly's Benjamin January series, Benjamin's friend and colleague, the violinist Hannibal Sefton, has relatively advanced pulmonary TB.

- Raistlin Majere of the high fantasy Dragonlance series is afflicted with a magical illness that closely mirrors tuberculosis.
- In A Tree Grows in Brooklyn Johnny Nolan's eldest brother Andy becomes ill and dies from "consumption".
- Beth March, the third daughter in Little Women, dies of tuberculosis after being weakened by a bout of scarlet fever.
- In Anne of the Island, the third book of the Anne of Green Gables series, Ruby Gillis, one of Anne's childhood friends, dies of "the galloping consumption."
- W. Somerset Maugham's short story Sanatorium, set in the north of Scotland, concerns the lives, deaths and outlooks of a series of tuberculosis sufferers.

Nonfiction

- Illness as Metaphor by Susan Sontag compares the metaphorical portrayal of TB to cancer.
- In his autobiography Angela's Ashes, Frank McCourt portrays the prevalence and impact of TB ("consumption") during his childhood in Ireland.
- Film:
- In the film Heavenly Creatures, directed by Peter Jackson, Juliet Hulme had TB, and her fear of being sent away 'for the good of her health' played a large role in determining the subsequent actions of herself and Pauline Parker.
- In the film Moulin Rouge!, Nicole Kidman's character Satine dies of consumption at the end of her biggest performance.
- In the first Zatoichi movie, Ichi's opponent Hirate has TB, which causes him to wish to die fighting Ichi.
- In the film There Will Be Blood Daniel Day Lewis's character's brother allegedly died of TB.
- In the film The Others a few of the secondary characters die from TB.
- In the film Tombstone the character of Doc Holiday is referred to as a 'lunger', and TB motivates the characters actions throughout the film. He dies of consumption near the end of the film.
- In the 1936 film Camille Greta Garbo portrays Marguerite Gautier, who dies from tuberculosis.
- Drunken Angel, a 1948 film by Akira Kurosawa, is the story of a doctor (Takashi Shimura) who is obsessed with curing tuberculosis in his patients, including a young

yakuza (Toshiro Mifune) whose illness is being used by his organization as a biological weapon.

l. Active tuberculosis will kill about two of every three people affected if left untreated. Treated tuberculosis has a mortality rate of less than 5%. The standard "short" course treatment for tuberculosis (TB), is isoniazid, rifampicin(also known as rifampin in the US), pyrazinamide, and ethambutol for two months, then isoniazid and rifampicin alone for a further four months. The patient is considered cured at six months (although there is still a relapse rate of 2 to 3%). For latent tuberculosis, the standard treatment is six to nine months of isoniazid alone.If the organism is known to be fully sensitive, then treatment is with isoniazid, rifampicin, and pyrazinamide for two months, followed by isoniazid and rifampicin for four months. Ethambutol need not be used. BCG, or bacille Calmette-Guérin, is a vaccine for TB disease. This vaccine is not widely used in the United States, but it is often given to infants and small children in other countries where TB is common. BCG vaccine does not always protect people from getting TB. If you were vaccinated with BCG, you may have a positive reaction to a TB skin test. This reaction may be due to the BCG vaccine itself or due to infection with the TB bacteria. Your positive reaction probably means you have been infected with TB bacteria if
    - You recently spent time with a person who has active TB disease; or
    - You are from an area of the world where active TB disease is very common (such as most countries in Latin America and the Caribbean, Africa, Asia, Eastern Europe, and Russia); or
    - You spend time where TB disease is common (homeless shelters, migrant farm camps, drug-treatment centers, health care clinics, jails, prisons).
    - The BCG vaccine should be considered only for very select persons who meet specific criteria and in consultation with a TB expert.

m. In spite of fewer people in this country suffering with TB, it remains a serious threat, especially for HIV-infected persons. In fact, worldwide TB is one of the leading causes of death among people infected with HIV. People infected with HIV (the virus that causes AIDS) are more likely than uninfected people to get sick with other infections and diseases. TB is one of these diseases.
    - Without treatment, as with other opportunistic infections, HIV and TB can work together to shorten the life of the person infected.
    - Someone with untreated latent TB infection and HIV infection is **much more** likely to develop active TB disease during his or her lifetime than someone without HIV infection.
    - Among people with latent TB infection, HIV infection is the strongest known risk factor for progressing to active TB disease.
    - A person who has both HIV infection and active TB disease has an AIDS-defining condition.

The good news is that HIV-infected persons with either latent TB infection or active TB diseasecan be effectively treated. The first step is to ensure that HIV-infected persons get a test for TB infection and any other needed tests. The second step is to help the people found to have either latent TB infection or active TB disease get proper treatment. Rapid progression from latent TB infection to active TB disease can easily be prevented.

n. TB bacteria can become resistant to the medicines used to treat TB disease. This means that the medicine can no longer kill the bacteria. Resistance to TB drugs can occur when these drugs are misused or mismanaged. Examples include
   - when patients do not complete their full course of treatment;
   - when health-care providers prescribe the wrong treatment, the wrong dose, or wrong length of time for taking the drugs;
   - when the supply of drugs is not always available; or
   - when the drugs are of poor quality.

o. Antibiotic resistant bacteria are resistant to treatment by first line drugs and must be treated with second line drugs which are generally more expensive and must be used for longer periods of time. In addition to the increased difficulty in treating the disease, the patient remains infectious longer increasing the risk to the public and to healthcare workers. Multidrug resistant TB also appears in association with HIV infection and AIDS, further compromising the health and the immune system of these patients.

Part 4, Page 15 Questions i-viii (1-8) Suggested Answers

i. Tuberculosis combines one of the slowest division rates among bacteria with a hardy cell wall defense system which results in the 9 month treatment regimen.
ii. Obligate aerobe means that the TB bacterium requires a large amount of oxygen in order to grow.
iii. The cell wall of MTB has three major components: mycolic acids, cord factors, and Wax-D. The mycolic acid molecules are of primary interest due to their deadly qualities. Mycolic acids are unique to Mycobacterium and Corynebacterium. They are alpha branched saturated fatty acids with chain lengths as long as 80 carbons. Mycolic acids create a lipid shield, which protects against cationic proteins, lysozyme, and oxygen radicals of phagocytosis.
iv. The Gram Stain is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. MTB cannot be classified as truly gram positive or negative, because its cell wall is impervious to gram staining due to the high content of lipids, especially mycolic acid.
v. MTB is a facultative intracellular parasite, which means that it can reproduce inside or outside of host cells. As a result, MTB is able to survive within immune cells known as macrophages without being destroyed by phagocytosis. When infectious

particles reach the alveoli sacs in the lungs, macrophages phagocytose (engulf) the bacteria and clump together into granulomas in order to contain the infection. Although this process keeps 95 percent of TB infections from becoming activated upon bacterial entry, MTB is able to remain dormant for many years, thanks to its cell wall which provides resistance to lethal oxidation.

vi. The name tuberculosis comes from tubercles. These are small, hard lumps that form when the immune system builds a wall around the TB bacteria in the lungs. Once TB becomes active small rounded nodules known as tubercles form and create an environment in which MTB is unable to multiply. However, because of its cell wall, MTB can survive in the low pH and anoxic tubercles for long periods of time. These tubercles are surrounded by many inactivated macrophages in which MTB is able to replicate. Through this process, although the cell-mediated immune response is capable of destroying individual bacterium, it is also responsible for the growth of tubercles, which occurs as MTB replicates within and subsequently ruptures inactivated macrophages .

vii. The long period of antibiotic treatment, combined with a laundry list of side effects, which can include hepatitis, optic neuritis, and seizures, compels many patients to stop taking their medication after symptoms subside.

viii. Two types of drug resistant MTB strains are currently recognized. Multi-drug-resistant tuberculosis (MDR TB) is resistant to at least two of the four first-line drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol). Extensively drug resistant tuberculosis (XDR TB) is defined as resistant to isoniazid, rifampin and also to fluoroquinolone and at least one of three injectable second-line drugs. XDR TB has an estimated cure rate of only 30 percent in patients with an uncompromised immune system compared to a 95 percent cure rate of normal tuberculosis.

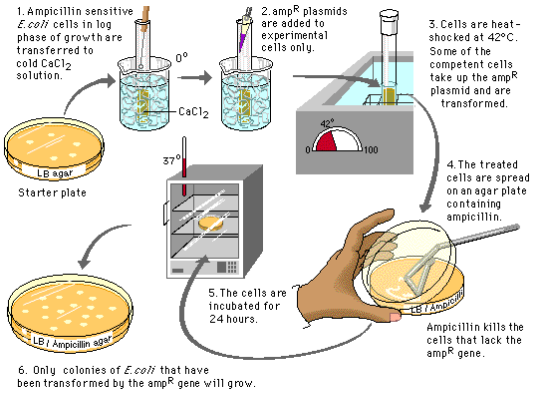
Part 4, Page 16 Questions i-v (1-5) Suggested Answers

i. *E. coli*

ii. A plasmid is a small ring of DNA that carries accessory genes separate from those of a bacterial chromosome. Also found in some eukaryotes, such as yeast.

iii. Wash hands and work surfaces; Keep Lid on petri dish; Open sterile tools carefully; Wear eye protection and keep hair pulled back, do not eat or drink in the lab, wash hands before leaving the lab.

iv.



v. The answers are:

Plate I – D
Plate II – B
Plate III – C
Plate IV – A

Part 5, Page 18 Suggested Rubric for Narrative Essays

	Points Possible		**Points Awarded**
Two 1st-person narratives (100)	**Narrative 1**	**Narrative 2**	
Well edited for spelling and grammar	**10**	**10**	
Good narrative structure: beginning, middle, end, point of view, Creative	**10**	**10**	
Narrative answers questions from assignment	**10**	**10**	
Accurate and complete information	**10**	**10**	
Much information about how the disease is communicated	**10**	**10**	
Total Points Possible (145)	**95**	**50**	

Name: _____ **Period: _____**

Date: _____

HIDING IN PLAIN SITE:

THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

Student Lab Guide – Introduction

As you are reading this laboratory guide your body is under attack! Pathogens (viruses, bacteria, protists and fungi) attempt to penetrate your protective outer coverings in an attempt to get a foothold and carry out their own life activities. When you think about it, your body is an ideal place for pathogens to grow and reproduce, as there is a rich supply of nutrients within a protected setting. These pathogens are everywhere, on every surface, in every environment. What's keeping them from infecting you is your immune system.

YOUR ASSIGNMENT:

In the first part of this 5-part unit, you will explore how a normal immune system functions to spare you the fate of an infection. You will learn the roles of the cells and organs of the body that make up the immune system. In later activities you will gather information concerning the actions of the immune system in fighting a specific battle against one type of pathogen, bacteria called *Mycobacterium tuberculosis.* This bacterium causes the disease tuberculosis. Through both a web tutorial and a simulated laboratory exercise you will learn how antibodies assist with immunity and with research and medicine. Then, you will investigate how bacteria mutate and gain resistance to the antibiotics used to treat people with bacterial infection. Finally, you will communicate your results by discussing what you have learned about the immune system, tuberculosis, and antibiotic resistance.

THE PLAN OF ACTION:

- Part 1 - Carry out the Immune System Web Tutorial
- Part 2 - Investigate Antibodies – Workhorses of Immunity and Research
- Part 3 - Carry out the Tuberculosis Web Investigation/Fact Sheet
- Part 4 - Conduct the Bacterial Transformation Lab
- Part 5 - Communicate your Results

To implement this plan please begin with **PART 1: IMMUNE SYSTEM WEB TUTORIAL**

Name: _____ **Period: _____**

Date: _____

HIDING IN PLAIN SITE:

THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

Student Lab Guide – Part 1 – Immune System Web Tutorial

IMPLEMENTING THE PLAN – PART 1 IMMUNE SYSTEM WEB TUTORIAL

1) Complete the Immune System Web Tutorial

The immune system consists of two main defensive strategies: Innate Immunity and Acquired Immunity. Innate Immunity consists of non-specific defenses such as the barrier defenses of skin and mucous membranes as well as the inflammatory response. This response is a coordinated action involving macrophages that ingest microbes, which penetrate barrier defenses; complement proteins that kill invaders and histamine and other chemicals that are released from injured cells which promote increased blood flow and blood vessel permeability at the injured area.

Acquired Immunity has two major features: antibody-mediated immunity and cell-mediated immunity. Both of these types of immunity rely on white blood cells called lymphocytes and their membrane bound receptors. In this tutorial you will review the cell components of a normal immune system.

a) We will be using web tutorials from two different publishers textbook companion websites during these exercises: Life: The Science of Biology by Sadava et. al, published by WH Freeman and Biology: A Guide to the Natural World by Krogh, published by Prentice-Hall

b) Go to The LifeWire Web site: http://bcs.whfreeman.com/thelifewire8e/ and select Chapter 18: Immunology from the drop down menu.

Figure 1. The Life Wire 8th Edition Web Site

c) Select “Animated Tutorials” from the left side menu and then select Tutorial 18.1 Cells of the Immune System

Figure 2. Chapter 18 Immunology Animated Tutorials menu page from The Life Wire 8th Edition web site

d) Read through the introductory material and then select the Chart Tab. Complete table 1 below. Be sure to click on each cell type graphic so that you can see an actual image of each cell.

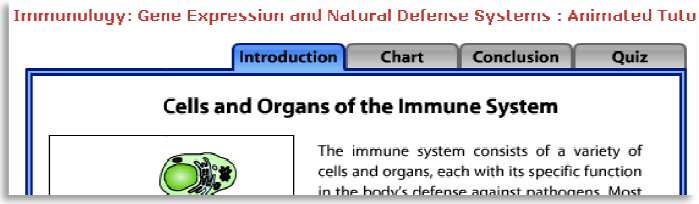
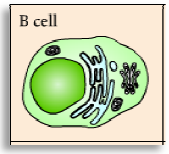
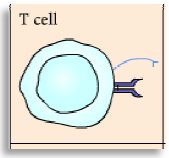
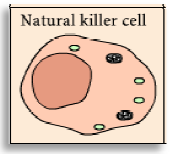
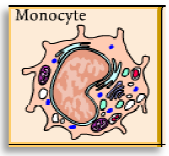
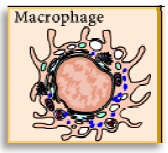
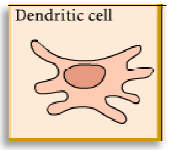
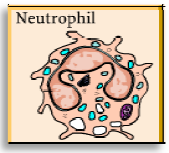
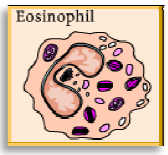
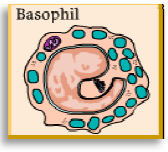


Figure 3. Cells and Organs of the Immune system from The Life Wire 8th Edition web site

TABLE 1. Cells of the Immune System. Complete this chart with information from The LifeWire web site.

CELL	CELL TYPE	FEATURES
		
		
		
		
		
		
		
		
		

e) Click on the "Organs" Tab and use the information presented to label the organs of the immune system on the body graphic (Figure 4) below.

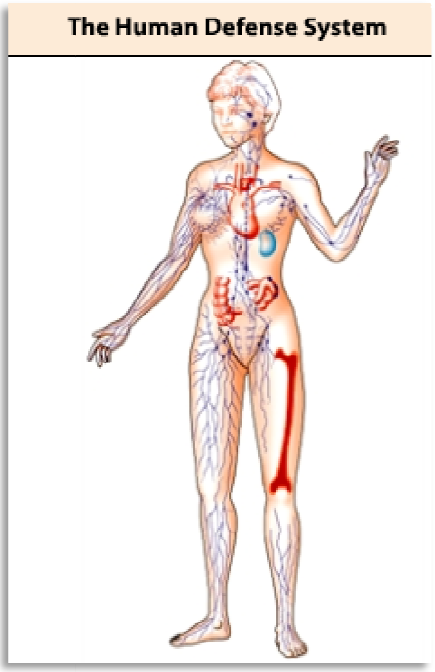


Figure 4. Chapter 18 Immunology Animated Tutorials page from The Life Wire 8th Edition web site

f) Read the conclusion tab and answer the questions below:

Questions 1 and 2.

How many white and red blood cells are there in a milliliter of blood?

_____

What is the role of lymph nodes in the immune system?

_____

_____

g) Use your browser's "back" button to return to the Animated Tutorials menu page (See Figure 2 on page 3.)

h) Select Tutorial 18.3 Humoral Immune Response

i) In the Humoral Immune Response, also called the antibody-mediated response, B Cells are activated to produce specific antibodies that circulate in blood and lymph in search of the specific pathogens that stimulated their activation in the first place. Answer the following questions as you work through the Tutorial:

Questions 3 through 7.

3. During the activation phase of the Humoral Immune Response, macrophages engulf and digest microbes, which get past the barrier defenses of the body. What do the macrophages do with the digested pieces?

_____

4. How do Helper T (T_H) cells become activated? What is Interleukin 1 and what is its role in (T_H) activation?

_____

_____

_____

5. What occurs in the next phase of the Humoral Immune Response?

_____

6. In the effector phase of the Humoral Immune Response what stimulates B cells to divide? What two types of cells result from B Cell activation?

_____

_____

_____

7. How are plasma cells adapted for antibody production?

_____

_____

j) Use your browser's "back" button to return to the Animated Tutorials menu page (See Figure 2 on page 3)

k) Select Tutorial Tutorial 18.4 Cellular Immune Response

l) The Cellular immune Response involves Natural Killer Cells, also called Cytotoxic T cells (T_C), that destroy target body cells, which are cancerous or have been infected by a virus. The Cellular immune Response also occurs in two stages. In the activation phase, T_C that have the correct T cell receptor are activated and divide. In the effector phase, these activated T_C cells encounter target cells and kill them. Answer the following questions as you work through the Tutorial:

Questions 8 through 11.

8. In the example shown in the Cellular Immune Response tutorial some of the virus proteins, which are antigens, are broken down by the cell and attached to Class I MHC proteins. These are then presented on the infected cell's cell surface. A Cytotoxic T cell (T_C), then participates in the next phase of the Cellular immune Response. What is unique about this Cytotoxic T cell?

_____

9. How do Cytotoxic T cells (T_C), become activated?

_____

_____

_____

10. Explain how these activated Cytotoxic T cells encounter and eliminate other body cells infected with this pathogen?

_____

_____

_____

11. How do Cytotoxic T cells distinguish between normal and abnormal body cells? Why aren't normal body cells attacked?

_____

_____

_____

_____

_____

YOU HAVE COMPLETED PART 1 IMMUNE SYSTEM WEB TUTORIAL → PLEASE CONTINUE ON TO STUDENT LAB GUIDE – PART 2 – ANTIBODIES – WORKHORSES OF IMMUNITY AND RESEARCH

Name: _____ **Period:** _____

Date: _____

HIDING IN PLAIN SITE:

THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

Student Lab Guide – Part 2 – Antibodies – Workhorses of Immunity and Research

IMPLEMENTING THE PLAN – PART 2 - INVESTIGATE ANTIBODIES – WORKHORSES OF IMMUNITY AND RESEARCH

2) Investigate Antibodies – Workhorses of Immunity and Research

An antibody or Immunoglobulin (Ig) is a soluble protein secreted by plasma cells. As you may recall from Part I of this activity, plasma cells arise from activated B Lymphocytes. Antibodies are Y-shaped antigen receptors. While antigen receptors, (Figure 1), found on B and T lymphocytes, have a transmembrane region, which anchors them to the cell surface, soluble antibodies (Figure 2) do not have this transmembrane region and thus are soluble in body fluids.

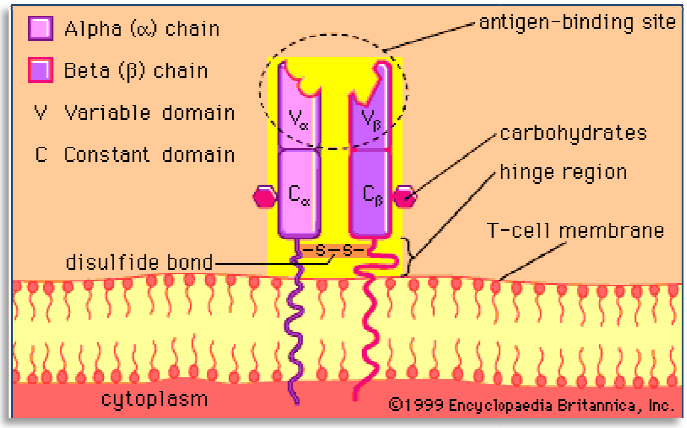


Figure 1. The basic structure of a T Cell antigen receptor

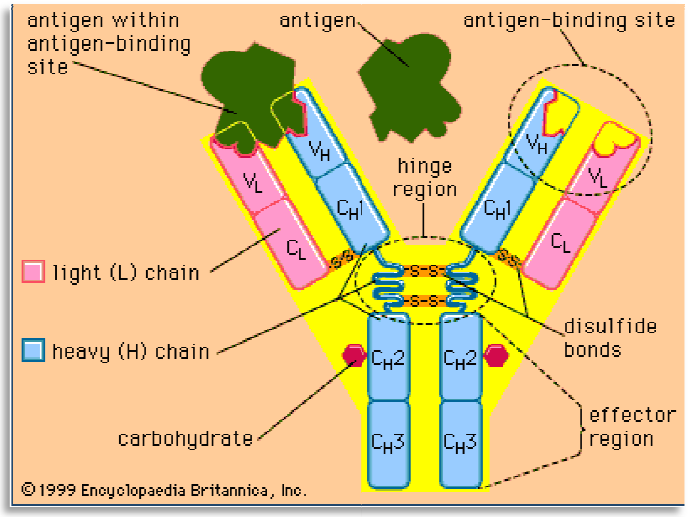


Figure 2. The structure of an antibody molecule binding with an antigen (green)

Any foreign molecule that is specifically recognized by B and T lymphocytes is called an antigen. Antigen receptors and antibodies recognize only a small portion of an antigen, called an epitope. A single antigen has many possible epitopes capable of inducing a response from a lymphocyte that recognizes it. Most antigens are large molecules such as polysaccharides or proteins. Many antigens stick out from the surface of pathogens such as bacteria.

All of the cell surface receptors on a single lymphocyte are the same. As such, they all recognize the same epitope and defend the body against any pathogen that produces molecules with that epitope.

In this investigation you will be researching and reporting on the various classes and uses of antibodies both by the immune system and in research and medicine. You will then investigate the laboratory-based assay ELISA (Enzyme-Linked Immunosorbent Assay) to experience how antibodies are useful in medical diagnosis.

a) Go the antibody guide website at ABCAM.com: http://www.abcam.com/index.html?pageconfig=tech

Figure 3. Abcam's antibody information webpage

b) Select part 1: The immune system and the antibody response and read through the information presented to complete the table and answer the questions that follow.

Questions 1 - 3.

1) How many classes of immunoglobulins do mammals have?

_____

2) Which immunoglobulins are the most abundant in mammals?

_____

3) Which immunoglobulin do all vertebrates share?

_____

TABLE 1. Immunoglobulin classes found in mammals. Complete this chart by sketching each immunoglobulin type and list its major characteristics

IMMUNOGLOBULIN CLASS	DRAWING	MAJOR CHARACTERISTICS

3) Conduct the Virtual ELISA at the HHMI Virtual Immunology Lab:

a) Go the Howard Hughes Medical Institute's virtual laboratory web site at http://www.hhmi.org/biointeractive/vlabs/immunology/index.html

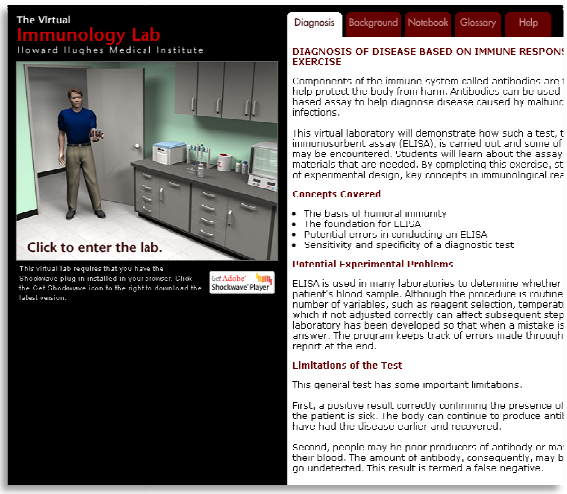


Figure 4. The HHMI Virtual Immunology Lab

b) Work your way through the virtual lab and answer the concept check questions below:

Questions 4 - 13.

4) What autoimmune disease does this virtual laboratory explore?

_____

5) What is serum and why was it loaded into the centrifuge? What was the 4th (purple top) tube used for?

_____

6) Why are serial dilutions necessary?

_____

7) All of the patient samples were placed on an ELISA plate. Describe the plate

_____

8) Why was the ELISA plate pretreated to bind SLE antigen in step 3? HINT: Click Why?

_____

9) What is the purpose of having both a positive and negative control?

_____

10) After incubation the plate is washed. What are you washing away?

_____

11) Why is rabbit anti-human antibody used?

_____

12) What is HRP-substrate? Explain how and why it is used

_____

13) Summarize the results of the ELISA plate. Which patient is likely to have SLE?

A	B	C	+	-

14) Write a description of the sandwich ELISA protocol as seen at right

A_____

_____

_____

_____

_____

_____

B_____

_____

_____

_____

_____

_____

C_____

_____

_____

_____

_____

_____

D_____

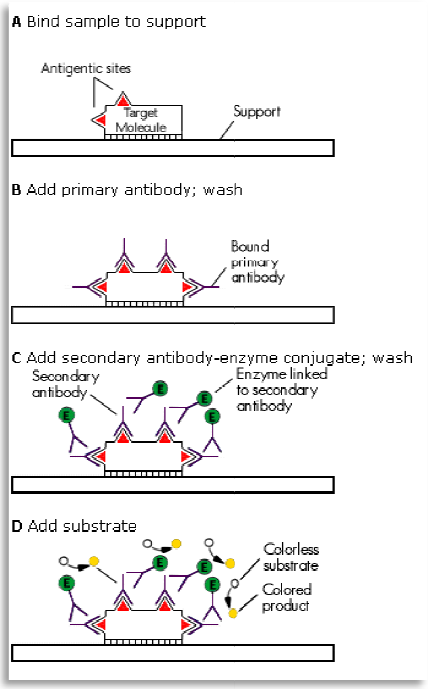
_____

_____

_____

_____

_____



4) Conduct the Simulated HIV ELISA According to your teacher's instructions.

5) **YOU HAVE COMPLETED PART 2 – ANTIBODIES – WORKHORSES OF IMMUNITY AND RESEARCH → PLEASE CONTINUE ON TO STUDENT LAB GUIDE – PART 3 - CARRY OUT THE TUBERCULOSIS WEB INVESTIGATION/FACT SHEET**

Name: _____ **Period: _____**

Date: _____

HIDING IN PLAIN SITE:

THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

Student Lab Guide – Part 3 – CARRY OUT THE TUBERCULOSIS WEB INVESTIGATION/FACT SHEET

IMPLEMENTING THE PLAN – PART 3 - CARRY OUT THE TUBERCULOSIS WEB INVESTIGATION/FACT SHEET

6) Investigate Tuberculosis

Tuberculosis, or TB, is a contagious disease caused by the *Mycobacterium tuberculosis* bacterium. Due to the wasting effects on its victims the ancient Greeks called the disease phthisis (pronounced TIE-sis) meaning 'wasting away.' In the last century, TB was known as consumption because it seemed to consume people from within. Once the leading cause of death in the United States, TB is still the leading cause of death in the world due to a single infectious agent. In 1995, 3 million people died from TB. According to the World Health Organization one third of the world's population is infected with TB bacilli and someone in the world is newly infected by the tuberculosis bacterium every second of every day.

a) Use your 21st century investigative research skills to prepare a fact sheet that answers the following questions about tuberculosis. Provide one paragraph for each question. Begin each paragraph with the question and then provide your answer based on what you found. End each paragraph with a reference, properly formatted, to the sources that you used to find the answer. [Extra credit will be given if you find and interview someone who had or has had tuberculosis or a medical professional who treats it.] (Modified from *You Make Me Sick: The Disease Project, Sir Francis Drake High School in San Anselmo, California)*

i) **The Basic Information Fact Sheet Questions**

(a) Who named/discovered Tuberculosis (TB)?
(b) What causes TB?
(c) Do any other organisms carry it or suffer from it?
(d) Is it associated with any particular region of the world? Why?
(e) How many people has it affected?
(f) Is everyone equally vulnerable, or does it target particular categories of people? Why?
(g) Is it chronic or acute?
(h) Is it infectious, and if so how easy is it to catch?
(i) What are the symptoms? How is it diagnosed?
(j) How does the virus, bacteria, gene, etc. interact with your body to cause the symptoms?
(k) Has the disease impacted the course of history? Give an example.

(l) Is there a cure or vaccine in mainstream medicine? What drugs/treatments are available?

(m) TB was virtually eliminated as a public health threat in the U.S. but then re-emerged in the 1990s especially in areas with high HIV infection. How are the two diseases related?

(n) Noncompliance with complete antibiotic treatment has lead to the emergence of multidrug resistant strains of TB. Give an example of how antibiotic resistance can evolve in strains of TB.

(o) Explain why we should all be worried about the emergence of antibiotic resistant strains of bacteria.

7) YOU HAVE COMPLETED PART 3 – CARRY OUT THE TUBERCULOSIS WEB INVESTIGATION/FACT SHEET ANTIBODIES – → PLEASE CONTINUE ON TO STUDENT LAB GUIDE – PART 4 - CONDUCT THE BACTERIAL TRANSFORMATION LAB

Name: _____ **Period: _____**

Date: _____

HIDING IN PLAIN SITE:

THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

Student Lab Guide – Part 4 – CONDUCT THE BACTERIAL TRANSFORMATION LAB

IMPLEMENTING THE PLAN – PART 4 - CONDUCT THE BACTERIAL TRANSFORMATION LAB

8) CONDUCT THE BACTERIAL TRANSFORMATION LAB

As you have just learned by researching tuberculosis and creating your Tuberculosis Fact Sheet a major problem is starting to materialize with the emergence of multi-drug resistant strains of Tuberculosis. In this activity, you will learn how TB, and bacteria in general, becomes resistant to antibiotics used to treat people who are infected with the pathogen.

a) Read the article *Antibiotic Resistance of Tuberculosis* found here: http://dujs.dartmouth.edu/winter-2009/new-trickes-for-an-old-foe-the-threat-of-antibiotic-resistant-tuberculosis

b) Answer the following questions

i) Name the two characteristics regarding the growth and anatomy of *Mycobacterium tuberculosis* (MTB) that results in a required 9-month treatment with antibiotics to cure an infected person.

_____

_____

ii) MTB is an obligate aerobic bacterium. What does obligate aerobe mean?

_____

iii) Describe the cell wall of MTB

_____

iv) What is the Gram Stain? Is MTB Gram positive or negative?

_____

_____

v) How is MTB able to survive inside of immune cells?

_____

_____

_____

vi) What are tubercules?

_____

vii) What compels TB patients to stop taking their medication?

_____

_____

viii) Describe the two types of drug resistant MTB strains.

_____

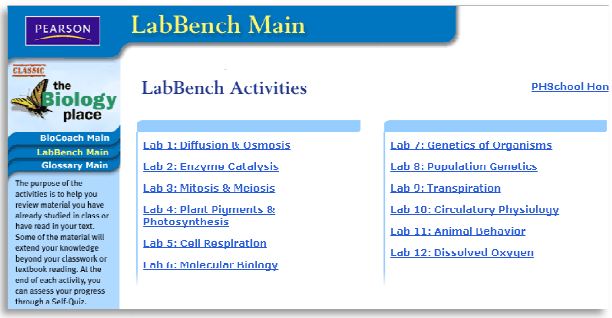
_____

_____

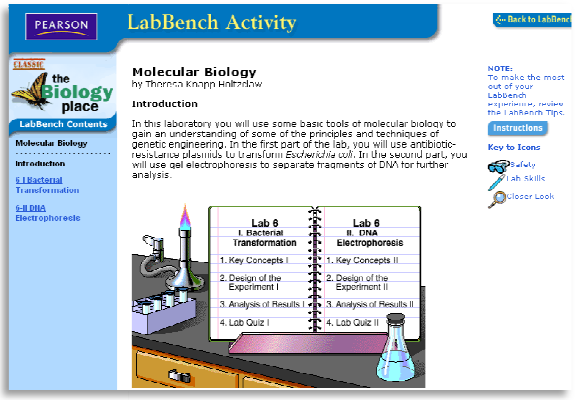
9) PREPARE FOR STEP 3 BY SIMULATING THE TRANSFORMATION LAB

a) Go to the Biology Place web site: http://www.phschool.com/science/biology_place/labbench/

b) Select Lab 6 Molecular Biology



c) Select activity 6-1 Bacterial Transformation, work through the tutorial and answer the questions that follow.



i) Instead of using *Mycobacterium tuberculosis* (MTB) this simulation uses what species of bacteria commonly found in the human gut?

_____

ii) What is a plasmid?

_____

iii) List a few of the sterile practices you should employ when working with bacteria in the lab

_____

_____

_____

iv) Outline the basic procedure below in a step-wise fashion:

_____

_____

_____

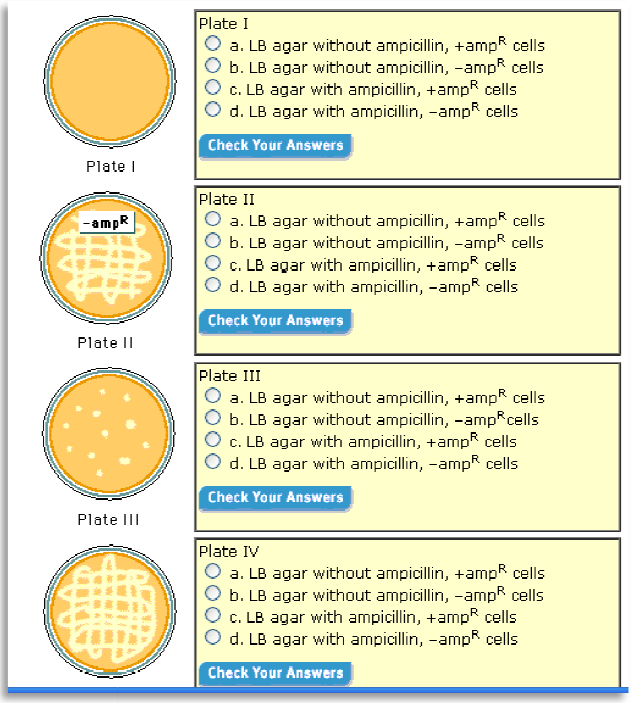
_____

_____

_____

_____

v) What should your results look like? Check the correct answers below



10) CONDUCT THE BACTERIAL TRANSFORMATION LAB ACCORDING TO YOUR TEACHER'S INSTRUCTIONS.

11) YOU HAVE COMPLETED PART 4 – CONDUCT THE BACTERIAL TRANSFORMATION LAB → PLEASE CONTINUE ON TO THE LAST SECTION OF THIS ACTIVITY STUDENT LAB GUIDE – PART 5 –COMMUNICATE YOUR RESULTS

Name: _____ **Period: _____**

Date: _____

HIDING IN PLAIN SITE:

THE IMMUNE SYSTEM, TUBERCULOSIS, AND ANTIBIOTIC RESISTANCE

Student Lab Guide – Part 5 –COMMUNICATE YOUR RESULTS

IMPLEMENTING THE PLAN – PART 5 –COMMUNICATE YOUR RESULTS

1) COMMUNICATE YOUR RESULTS

Now it is time to wrap up your study of the immune system, Tuberculosis, and antibiotic resistance. To communicate what you have learned you will be writing two narrative essays from two very different perspectives:

a. You are to write a 3-4 page story in the first person (call the protagonist "I") describing yourself as one who contracts tuberculosis, suffers from the disease and then begins a regimen of antibiotics to cure the disease. Half way through the 9-month antibiotic regimen, you've had enough and you decide to stop taking your medication. Explain how and when you first knew something was wrong and why you decided to stop taking the medication. Describe the progress of your symptoms, how it feels, how it has affected your daily life, and how your family and friends have responded. Next, describe your hopes and fears for the future. ACCURACY and completeness (did you get all the facts right?) and also quality of writing, creativity and pathos (noble suffering) count towards your grade.

b. Now, write the same story (3-4 pages) from the point of view of the germ! What do you as the cause of the disease hope to accomplish? What is your experience inside the human body? How did you get there? Was it easy or difficult? Did you mean to do it? What are your worries and concerns regarding the immune system? (Did a macrophage eat any of your friends?) Describe yourself and explain how you try to evade the host's immune system. Describe how you become resistant to the antibiotics that your host has stopped taking. You can anthropomorphize shamelessly. This means you can write as though you are the disease and explicitly describe what you are purposively thinking and planning to do within the host.

(Modified from You Make Me Sick: The Disease Project, Sir Francis Drake High School in San Anselmo, California)

2) YOU HAVE COMPLETED THIS UNIT ON THE IMMUNE SYSTEM