Overview of the Immune System and Immunological Assays with Real World Connections

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A. Teacher Guide

I. Background:

i. Introduction to Immunology Part 1 and Cell Research Project

What is our immune system? What is immunity? What are we trying to protect ourselves against? Those are the questions that many students will ask. This unit on immunology was created to answer these basic questions with videos, notes, labs, research projects, presentations, and collaborative work. Students will learn the difference between innate and adaptive immunity. Innate immunity is the fast acting, non-specific immune system that acts against a broad range of pathogens, but if that is not enough, it triggers the adaptive immune system to help fight the pathogens. The adaptive immune system takes longer to activate because it uses very specific antibodies and receptors against varying pathogens and antigens. The following link is to the National Institute of Allergy and Infectious Disease and is a great place to start to learn about the immune system: https://www.niaid.nih.gov/topics/immunesystem/Pages/default.aspx

There are a variety of WBC's (white blood cells) in your immune system that help protect you against pathogens that are constantly attacking your body. You can think of your immune system as the military for your body, and there are different branches that have different functions to protect us. The students will research and become experts on one assigned cell of their body's immune system. They will then make a presentation that they will use to teach the class about their assigned cell. You can find more information about the specifics of each cell type at the following link: https://www.niaid.nih.gov/topics/immuneSystem/Pages/immuneCells.aspx

ii. Introduction to Immunology Part 2 and Vaccines Article

As the students start to learn about their immune system, you will introduce the topic of vaccines. You may ask students how do vaccines work? What causes allergies? Why can't I take antibiotics to treat a viral infection? What is MRSA? This part of the unit will focus on answering these questions. You will answer some of these questions in this part of the lesson.

A vaccine typically contains an agent that resembles a disease-causing micro-organism and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. Improving vaccinations and creating new vaccinations is a hot topic in the research community right now. Medicine is

always changing and evolving due to new discoveries completed by researchers. In 1998, a study was published by Dr. Andrew Wakefield that tied autism to the MMR (measles, mumps, and rubella) vaccine. This was later retracted because of lack of replication and a conflict of interest that arose during the study. More information about this topic can be found at the following link: http://www.cnn.com/2011/HEALTH/01/05/autism.vaccines/index.html.

But, researchers have been trying to find a way to create more powerful vaccines that will have longer protection with less harmful side effects for their recipients. Vaccines actually inject inactivated viruses, parts of the pathogens DNA, or a variety of other antigens specific to the pathogen of interest. Currently, many vaccines also need booster shots to enable your body to maintain appropriate levels of antibodies in your serum to provide a protective effect against the pathogen. If we could find a way to decrease the amount of injectable pathogen/antigen while increasing the initial immune response to provide a stronger, longer lasting immunity that would be a great leap in the medical field. They are also trying to develop vaccines that would treat rapidly mutating viruses like HIV, but we haven't gotten there yet.

The purpose of this lesson is to have students practice reading and dissecting research articles. They will also have to present their article findings to the class and lead a class discussion on the benefits and/or complications of the study. These articles are all tied to vaccinations so that it ties into the next activity (the ELISA lab on vaccination methods), but can be changed based on your needs. They will use these articles to jump start their project idea on comparing vaccination methods that will be discussed in the ELISA protocol.

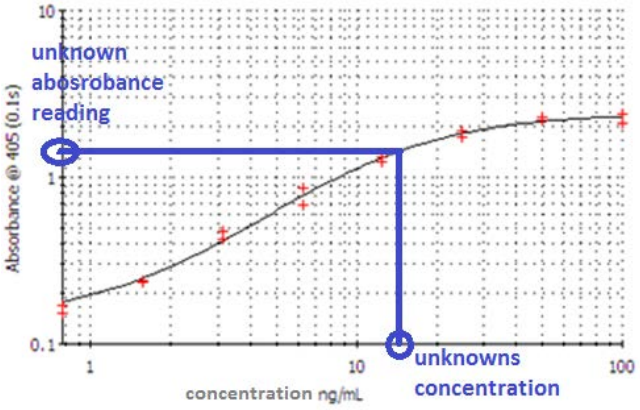
iii. Quantitative ELISA Lab

ELISA stands for Enzyme Linked Immunosorbent Assay. ELISA's are a common immunological assay with a variety of uses, such as to check antibody titers when giving someone a booster of tetanus, when checking for the presence of an antigen in serum (such as malaria or HIV), when checking the presence of a specific antibody in serum (like for allergens or against other specific antigens), and many other things. Your body's immune cells make antibodies against the specific antigens. ELISA's are run to detect either the antibody or the antigen in your serum.

To detect these, ELISA's use antibodies that are linked to enzymes to detect antigens in a patients' serum. The enzyme that is attached to the antibody

produces a visible color change when a substrate is added in its presence. A substrate is something that binds to the enzyme that the enzyme then acts on to change it in some way. If the antigen is not in the sample (which could be another antibody, a protein, a virus, etc.), the antibody that is linked to the enzyme will not be able to bind to anything, so it will be washed away. When the substrate is added, there will be no color change. If the there is a color change, that means that your enzyme linked antibody was able to bind to its specific target antigen in the well. You can determine the qualitative concentration of the antigen present in the well by observing the intensity of color change. The darker the color change, the more you have. If you have standards that you serially dilute at 1:2 of a known concentration, you can determine the exact quantity of antigen present in your well. You can reference the attached ELISA PowerPoint to obtain more details about the assay. It has links to helpful videos as well.

Knowing the difference between a qualitative and quantitative assay is crucial in this lesson because you will be combing both types on one plate. Qualitative assays give you a relative concentration of antibody or antigen present in your serum in comparison to other samples. Quantitative assays allow you to determine the exact concentration of antigen or antibody present in your sample. The Quantitative assay uses standards, which have known concentrations, to generate a graph against the obtained absorbance values. Absorbance is the ratio of incidence to transmission of radiant energy (light) through a material. These values are placed on a log scale graph (this is to present a wide range of data in a more compact area) to generate a standard curve like the one above. You can then use that curve as a reference to determine unknown sample concentrations when given the absorbance of your unknown sample by your plate reader. For example, if your unknown sample had an absorbance reading of 1.5 nm, your concentration would be about 10.5 ng/ml.



iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

HIV stands for Human Immunodeficiency virus. It is a virus that attacks helper T-cells (Th cells), also known as CD4 T-lymphocytes, in your immune system. Th cells are also called CD4 T-cells because they have CD4 receptors on their plasma membrane surfaces. CD4 T-cells have roles in both the humoral and cell mediated immunity in the adaptive branch of the immune system as seen in figure 1 below. With lower levels of CD4 T-cells, the adaptive immune system will struggle to mount an appropriate antibody mediated response or cytotoxic T-cell response to new infections, leaving the infected individual more susceptible to opportunistic infections and cancer.

https://www.intechopen.com/source/html/39824/media/image2.jpg

Figure 1

The HIV virus is coated with a gp41 (glycoprotein-41) and gp120 (glycoprotein-120) viral envelope complex. The looped domains on gp120 are recognized by the CD4 TCR (T-cell Receptor) and CCR5 (Chemokine co-receptor 5) or CXCR4 (chemokine co-receptor 4) (figure 2). When gp120 binds with CD4 and a co-receptor, conformational changes occur in the membrane proteins, which bring the virus toward the cell, allowing their membranes to fuse. Once membrane fusion occurs, the contents of the virus are released into the cell. Viral RNA is reverse-transcribed into DNA and gets integrated into the T-cells DNA. The T-cell then uses its own resources, such as ribosomes, nucleotides, amino acids, etc. to transcribe and translate viral DNA into necessary proteins, additional viral RNA, etc. to assemble more viruses. As these materials are constructed, they gather at the cell membrane to ready themselves for viral budding. Viral budding occurs once all viral materials are present and they exit the cell by utilizing the T-cells plasma membrane as its new viral envelope, being released to infect other CD4 T-cells. Once enough viruses bud from the T-cell, the plasma membrane integrity becomes compromised, causing T-cell death to the infected cell (figure 3).

https://image.slidesharecdn.com/9bio265virusesfungiprotozoahelminthsinstructordrdibonaventura-141204212337-conversion-gate02/95/9-bio265-viruses-fungi-protozoa-helminths-instructor-dr-di-bonaventura-13-638.jpg?cb=1417728675

Figure 2

https://www.intechopen.com/source/html/16788/media/image3.jpeg

Figure 3

Healthy, uninfected individuals, usually have a range of CD4 T-lymphocyte between 500-1,600 cells/mm^3. This number varies based on sex, age, ethnicity, and a variety of other factors unrelated to HIV infection. This number substantially drops as increased numbers of CD4 T-lymphocytes are infected and eventually undergo cell death. AIDS, which stands for Acquired Immunodeficiency Syndrome, occurs when your CD4 T-lymphocyte count drops below 200 cells/mm^3. With such a low number of CD4 T-cells, your immune system struggles to protect itself from opportunistic infections or cancer. HIV doesn't directly kill a person, it's the other opportunistic infections or cancer that end up causing death of the HIV infected individual.

Because the number of CD4 T-lymphocytes varies greatly between individuals, when diagnosing an individual with AIDS, CD4 cell counts, CD4 percentages, and CD4:CD8 T-cell ratios are reviewed. Percentages of the lymphocytes and their ratios tend to be more stable overtime than actual cell counts. Reference ranges of healthy individuals for cell counts, percentages, and ratios are listed in the attached worksheets. We can determine the cell counts, percentages, and ratios by analyzing serum samples on a flow cytometer.

A flow cytometer is an instrument that can analyze cells with a laser based on size, granularity, and color of fluorescence. The cytometer can detect cell size by looking at how much the laser light scatters in the forward direction when the cell passes through the beam (figure 4). The cytometer can detect granularity by detecting the amount of laser light that scatters in the side direction (figure 5). Cells can be stained with fluorescent molecules attached to antibodies that are specific for different cells surface receptors (such as CD4 and CD8), with internal fluorescent proteins (such as GFP- green fluorescent protein), or internal fluorescently tagged molecules (such as CFSE- Carboxyfluorescein Succinimidyl Ester**)**.

http://1.bp.blogspot.com/-Rj9IGvNRoZg/VT81paixhnI/AAAAAAAAEJQ/U51pbBgtdkE/s1600/5.jpg

Figure 4

https://aws.labome.com/figure/te-125-4.png

Figure 5

When fluorescently tagged antibodies attach to the cell receptors, their electrons move to an excited state as they pass through the laser light in the cytometer. The excited fluorochrome releases light at a particular wavelength as their electrons fall back to their ground state (figure 6). Different wavelengths of released light are detected by different PMT's (photomultiplier tubes) that have different filters in front of them. This allows the use of multiple antibodies which are specific for different cells markers to be used at the same time, as long as they have attached fluorochromes that are detected by different PMT's (figure 7). Most cytometers can analyze 4 colors at one time, and utilize a red (635 nm wavelength) and a blue laser (488 nm wavelength) to do so, but some instruments can go up to 18 colors at a time (such as the LSR II) using up to 4 different lasers.

http://www.antibodies-online.com/images/news/Fluorescence.png
Figure 6
https://upload.wikimedia.org/wikipedia/commons/thumb/3/3f/Cytometer.svg/800px-Cytometer.svg.png
Figure 7

Results from the cytometer can be recorded on multiple plots. Cell size and granularity is shown on dot plot of forward vs. side scatter. Cells are represented by dots on the plot. Small, low granularity cells are located toward the bottom left corner of the plot (such as lymphocytes) and large, high granularity cells are located in the top right of the plot (such as granulocytes) figure 8). Fluorescence can be displayed in both dot plots and/or histograms. Histograms display fluorescent intensity signals only collected by one detector (one color) against relative cell counts, whereas dot plots can display 2 colors at a time (figures 9 and 10 respectively). This can help differentiate between single positive or double positive populations. You can do more complex analysis of triple positive populations by applying various gating methods to your dot plots, but that will not be discussed in detail.
http://flowcytometry.weebly.com/uploads/2/4/2/9/24290417/2773370.jpg
Figure 8

Students are going to learn about flow cytometry from the Power Point presentation and the embedded video links. They will be briefly assessed at different points during the presentation by answering various questions throughout the presentation. Afterwards, they will act as the flow cytometer to determine various WBC counts and percentages present in their patients' serum

sample. Additionally, they will utilize their knowledge of HIV/AIDS and given reference ranges on their worksheets to determine if their HIV positive patients should be officially diagnosed with AIDS. During this diagnosis process they will fill out the "HIV/AIDS Testing –Sample Transit, Receipt, and Reporting Form" to act as true biotechnicians in the classroom. Finally, they will complete the "Flow Cytometry Raw Data Form" for 2 different cell types that were given in their patient serum sample. Both worksheets act as an assessment method for their comprehension of flow cytometry before the summative assessment at the end of the unit.

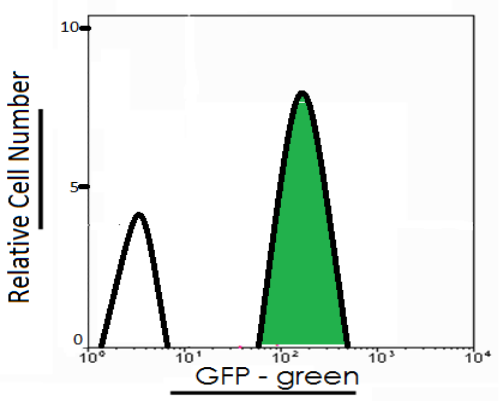


Figure 9

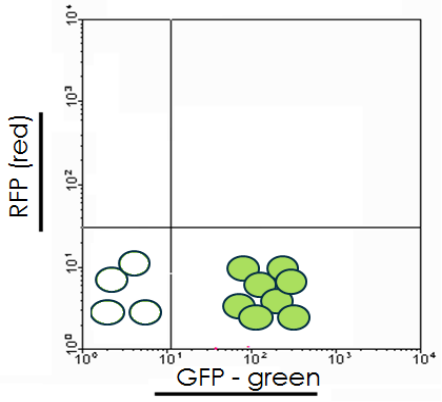


Figure 10

References:

1. Alimonti, Judie B., Blake Ball, and Keith Fowke. "Mechanisms of CD4 T Lymphocyte Cell Death in Human Immunodeficiency Virus Infection and AIDS." Weblog post. *Microbiology Society*. Journal of General Virology, 07 Jan. 2003. Web. 11 Aug. 2016.
2. "HIV Basics." *HIV/AIDS*. Centers for Disease Control and Prevention, 06 July 2016. Web. 11 Aug. 2016.
3. "Types of Lab Tests." *HIV/AIDS Basics: Just Diagnosed with HIV AIDS: Understand Your Test Results*. AIDS.gov, 11 Oct. 2010. Web. 11 Aug. 2016.
4. "Introduction to Flow Cytometry: A Learners Guide." BD Biosciences, Apr. 2000. Web. 11 Aug. 2016.

v. Antibiotic Resistance Assay

Antibiotics are a type of antimicrobial substance that specifically targets pathogens that can cause infection and disease in individuals. Antibiotics came into medical use in the early 1940's and have seen growing use since then. Due

to the increased and widespread use of antibiotics, the pathogenic microbes that these drugs initially targeted have evolved to evade the antibiotic mechanisms that originally caused their demise. For example, MRSA (methicillin-resistant staphylococcus aureus) evolved to evade the entire class of penicillin-like antibiotics called beta-lactams. MRSA has acquired a plasmid that contains the *mecA* gene, which codes for the PBP2a (penicillin binding protein 2a), causing the penicillin-like antibiotics to become ineffective at inactivating the bacterial enzyme transpeptidase. Transpeptidase is an enzyme that is crucial for bacterial cell wall synthesis. Since the bacterial cell wall synthesis is not hindered when the bacteria contains the *mecA* gene, it can go on dividing and conquering your body. There are many other dangerous antibiotic resistant organisms out there that have developed multiple mechanisms of resistance for multiple types of antibiotics. Figure 1 shows a list of different antibiotics and their general targets within the pathogen. Figure 2 shows the multiple mechanisms that pathogens can use to evade those antibiotics.

https://pharmacologydeh-28classof2011.wikispaces.com/file/view/antibiotic_targets_web.gif/140603825/564x413/antibiotic_targets_web.gif

Figure 1

http://textbookofbacteriology.net/ResistanceMechanisms.gif

Figure 2

How do microorganisms that once were antibiotic sensitive gain resistivity to the same antibiotics? Genes for resistivity can be acquired through internal gene mutations, lateral gene transfers from another bacterium, or infection by a bacteriophage (a virus that attacks bacteria).

As of 2013, there were 18 different drug resistant organisms that the CDC identified as threatening to the US population. These drug resistant organisms are broken down into three categories: urgent threats, serious threats, and concerning threats. Three organisms fall into urgent threats, twelve into serious threats, and three into concerning threats. As time and antibiotic use continues, there will undoubtedly be an increase in the number of antibiotic resistant organism. Drug companies are feverishly trying to develop and discover new antibiotics and antimicrobial agents to solve the problem of antibiotic resistance.

Most outbreaks of MRSA and other antibiotic resistant infections occur in hospitals or medical facilities where there are high concentrations of patients with weakened/compromised immune systems or open wounds. Resistant organisms are found in these places more readily because of the widespread use of antimicrobials and antibiotics. Selective pressure in the environment favors antibiotic and antimicrobial resistance because they are used to clean surfaces, wounds, etc. all the time.

When outbreaks occur in these settings there needs to be rapid action taken to address the concern. If an immunocompromised person is infected with one of these pathogens, it can be life threatening. Since there are many patients in close proximity, disease can spread easily, which increases the need for a quick response to the outbreak. One way to quickly address the outbreak is to culture the bacteria and test multiple antibiotics or antimicrobial agents on the organism at the same time. This is called an antibiotic resistance assay. To test the resistance or sensitivity of an organism to different antimicrobial agents, you need to first inoculate the plate with the organism of interest and then soak small filter paper disks in the antimicrobial agents of interest and place them on the inoculated dish. The dish has to then be incubated overnight to observe growth (or the inhibition of growth)

Figure 3 shows a petri dish setup for an antibiotic resistance assay for *e. coli* bacteria. The small white circles are the filter disks that are soaked in different antimicrobial agents. The cloudy white region on the plate is the bacterial lawn growth. The clear region around each small filter disk is the zone of inhibition, which is where the bacteria did not grow because it was inhibited by the antimicrobial agent. The larger the zone of inhibition around the filter disk, the more effective the agent was at inhibiting growth.

https://nanowatertreatment.wikispaces.com/file/view/Disk_Diffusion_Assay_2Q2025.jpg/275338054/Disk_Diffusion_Assay_2Q2025.jpg

Figure 3

References:

1. www.CDC.gov/drugresistance/about.html
2. https://www.niaid.nih.gov/research/antimicrobial-resistance

vi. Ouchterlony Assay Development

Ouchterlony Assays are used to determine if patient serum contains antibodies against particular antigens, specifically, allergens. Antigens may be anything from surface proteins on viruses and bacteria to free-floating proteins or carbohydrates. The free-floating molecules are usually the ones that induce an allergic response. An allergic response occurs when the antigen being recognized induces an IgE response. IgE antibodies stimulate inflammatory responses, such as swelling, redness, and itchiness.

When a single antibody binds an antigen, it is too small to be seen. But, when hundreds of antibodies bind hundreds of antigens, the reaction is visible on an Ouchterlony plate. The reaction appears as a white or colored line on the plate between the patient serum sample and the antigen that causes the allergic response (aka the allergen). This binding reaction between the antibody and the allergen is called agglutination, which means clumping. It is because of this agglutination property that makes the reaction visible to the naked eye. If it were just hundreds of single antibodies bound to antigens, it would not be visible, but since they all clump together it becomes visible.

So how do you set up an Ouchterlony Assay? First, you fill a petri dish with agarose and let it solidify. Second, you use a 3 ml transfer pipet that is cut halfway up from the bottom to form wells in the agar. To do this you squeeze out all the air in the bulb of the pipet, then you stick the end of the pipet into the agar and release the pressure on the bulb of the pipet. When the pressure is released, the agar should get sucked into the pipet, leaving a perfectly shaped circular well in the agar. You will repeat this to make as many wells in your agar as desired for your assay. You should make your first well in the center of the plate for your patients' serum sample to go into. Suspected allergens will go in the surrounding wells.

Once the samples are added to their appropriate wells, the serum and the allergen suspensions diffuse out of their wells into the surrounding agar. The allergen and the serum will eventually run into each other. If there are antibodies in the patients' serum that are specific to the allergen in the nearby well, a precipitation line will form in the media. This line of precipitation is formed from the agglutination (clumping) of the allergens by the antibodies. If there are no antibodies specific for the allergen in suspension, then no precipitation line will form because there will be no bonding of the antibodies to the allergens.

Ouchterlony tests can not only be used to test for allergies, but also to see if a person has had prior exposure to an antigen (such as certain strains of the flu virus), to test for the presence of a particular antigen in your serum (such as HIV), or to test for antibody affinity to specific proteins. If you haven't learned already, there are multiple uses for multiple tests in immunology and some assays have overlapping uses. But, it is what you know and the type of data that you need to retrieve to help you pick the correct assay to perform.

II. Student Outcomes:

i. Science Concepts covered:

- Agglutination
- Allergens
- Allergies
- Analyzing data
- Antibiotic resistance
- Antibiotics and antibiotic resistance
- Antibodies
- Antibody specificity
- Antigens
- CD4 T-cells role in the immune system
- Concentration
- Culturing bacteria
- Determining unknown sample concentration
- Diagnostic testing
- ELISA's
- Experimental design
- Experimental execution
- Flow Cytometry
- Graphing standard curves
- HIV/AIDS
- Immune cell communication and interaction
- Immune Cell Development and location
- Immune Cell Types
- Immunity: innate vs. adaptive immune system
- Immunology assays
- Making working concentrations of antibiotics

- Measuring
- Ouchterlony assay
- Pathogens and antigens
- Patient Reports
- Vaccines

ii. Standards

- **NGSS Standards**

a. HS-LS1-1. All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.
b. HS-LS1-1. Systems of specialized cells within organisms help them perform the essential functions of life.
c. HS-LS1-2. Develop and use a model based on evidence to illustrate the relationships between systems or between components of a system.
d. HS-LS1-2. Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level.
e. HS-LS1-3. Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly.
f. HS-LS1-3. Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system.
g. HS-LS1-4. Models (e.g., physical, mathematical, computer models) can be used to simulate systems and interactions—including energy, matter, and information flows—within and between systems at different scales.
h. HS-LS1-6. Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.
i. HS-LS3-1. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways.
j. HS-LS3-2. In sexual reproduction, chromosomes can sometimes swap sections, thereby creating new genetic combinations and

thus more genetic variation.

k. HS-LS3-2. Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. Environmental factors can also cause mutations in genes, and viable mutations are inherited.
l. HS-LS3-2. Make and defend a claim based on evidence about the natural world that reflects scientific knowledge, and student-generated evidence.
m. HS-LS3-3. Environmental factors also affect expression of traits, and hence affect the probability of occurrences of traits in a population. Thus the variation and distribution of traits observed depends on both genetic and environmental factors.
n. HS-LS3-3. Technological advances have influenced the progress of science and science has influenced advances in technology.
o. HS-PS4-2. Engineers continuously modify these technological systems by applying scientific knowledge and engineering design practices to increase benefits while decreasing costs and risks.
p. HS-PS4-2. HS-PS4-5. Information can be digitized (e.g., a picture stored as the values of an array of pixels); in this form, it can be stored reliably in computer memory and sent over long distances as a series of wave pulses.
q. HS-PS4-4. HS-PS4-5. Multiple technologies based on the understanding of waves and their interactions with matter are part of everyday experiences in the modern world (e.g., medical imaging, communications, scanners) and in scientific research. They are essential tools for producing, transmitting, and capturing signals and for storing and interpreting the information contained in them.
r. HS-PS4-5. Science and engineering complement each other in the cycle known as research and development (R&D).
s. HS-ETS1-1. Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants.
t. HS-ETS1-3. Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics as well as possible social, cultural, and environmental impacts.

- **Introduction to Biotechnology: Georgia Standards**

a. HS-IBT-4. Students will demonstrate how concepts of physical science connect to biochemical applications and techniques.
   - Analyze enzyme activity using assays for reactants and products.
   - Utilize electrophoresis, chromatography, microscopy and spectrophotometry to identify, separate and to draw conclusions about biological molecules.
   - Use antibody specificity for antigens to test for the presence of protein (e.g., ELISA, Western Blot, antibody staining).
   - Utilize electrophoresis, chromatography, microscopy and spectrophotometry to identify, separate and to draw conclusions about biological molecules.
   - Calculate and prepare buffers, stock solutions, and reagents.

b. HS-IBT-5. Students will compare and contrast common organisms used in biotechnology and relate the manipulation of living organisms to product and procedure development.
   - Describe the characteristics and life cycles of model organisms used in biotechnology, including bacteria (e.g., *E. coli*), fungi (e.g., yeasts and *Aspergillus*), and animals (e.g., *C. elegans,* fruit flies, and rodents).
   - Monitor how environmental factors affect the growth of cells and model organisms in the laboratory.
   - Apply the basic concepts of cell growth to manipulate cultures under aseptic conditions in the laboratory.
   - Perform transformations, including competency, selection, antibiotic resistance, and analysis of transformation efficiency.

c. HS-IBT-7. Students will analyze economic, social, ethical, and legal issues related to the use of biotechnology.
   - Differentiate between moral, ethical, and legal biotechnology issues.
   - Research ethical issues presented by evolving science, including genetically modified foods, cloning, bioterrorism, gene therapy, and stem cells.
   - Compare and contrast attitudes about the use of biotechnology regionally, nationally, and internationally.

d. SCSh1. Students will evaluate the importance of curiosity, honesty, openness, and skepticism in science.

- Exhibit the above traits in their own scientific activities.
- Recognize that different explanations often can be given for the same evidence.
- Explain that further understanding of scientific problems relies on the design and execution of new experiments which may reinforce or weaken opposing explanations.

e. SCSh2. Students will use standard safety practices for all classroom laboratory and field investigations.
   - Follow correct procedures for use of scientific apparatus.
   - Demonstrate appropriate technique in all laboratory situations.

f. SCSh3. Students will identify and investigate problems scientifically.
   - Collect, organize and record appropriate data.
   - Graphically compare and analyze data points and/or summary statistics.
   - Evaluate whether conclusions are reasonable by reviewing the process and checking against other available information.
   - Develop reasonable conclusions based on data collected.

g. SCSh4. Students use tools and instruments for observing, measuring, and manipulating scientific equipment and materials.
   - Develop and use systematic procedures for recording and organizing information.
   - Use technology to produce tables and graphs.
   - Use technology to develop, test, and revise experimental or mathematical models.

h. SCSh5. Students will demonstrate the computation and estimation skills necessary for analyzing data and developing reasonable scientific explanations.
   - Consider possible effects of measurement errors on calculations.
   - Express appropriate numbers of significant figures for calculated data, using scientific notation where appropriate.
   - Solve scientific problems by substituting quantitative values, using dimensional analysis and/or simple algebraic formulas as appropriate.

i. SCSh6. Students will communicate scientific investigations and information clearly.

- —Write clear, coherent laboratory reports related to scientific investigations.
- —Write clear, coherent accounts of current scientific issues, including possible alternative interpretations of the data.
- —Use data as evidence to support scientific arguments and claims in written or oral presentations.
- —Participate in group discussions of scientific investigation and current scientific issues.

j. SCSh8. Students will understand important features of the process of scientific inquiry. Students will apply the following to inquiry learning practices:

- —Scientific investigators control the conditions of their experiments in order to produce valuable data.
- —Scientists use practices such as peer review and publication to reinforce the integrity of scientific activity and reporting.

iii. Placement:

This unit of instruction is appropriate for a high school Biotechnology course, AP Biology, or any other type of Life Science Elective that introduces Immunology.

iv. Skills:

Students will run various immunological assays to gain first-hand experience making reagents, using equipment (ex. Centrifuges, ELISA plate readers, etc.), gathering information from published sources, running assays, analyzing data, generating graphs in Excel, and keeping a scientific notebook.

v. Relevance:

The immune system is a vital system in your body that protects your body and keeps it healthy. Students should learn what their immune system is and how it functions in order to stay healthy. They should also be well informed about assays that can be run in case they have any types of immunological issues.

III. Student Learning Objectives

- **Students will be able to:**

a. Classify immune cells based on the branch of the immune system that they participate in and their major function.
b. Analyze anatomical barriers in the innate immune system.
c. Compare the immune system branches (innate or adaptive), and explain the functions of each.
d. Research information about a specific immune cell.
e. Communicate their understanding by creating a presentation about a specific immune system cell.
f. Teach the class about their assigned cell and ensure student understanding.
g. Construct a diagram of immune cell interactions.
h. Read, gather, summarize, and present overall from the assigned scientific text about recent vaccination advances.
i. Cite specific textual evidence to support their overall summary of the scientific text that relate to procedures and data obtained during the study.
j. Reflect on the procedure and data in the published work to see if there are any ways to improve the study.
k. Reflect on the study and determine how these results affect the bigger picture of our body.
l. Propose a study that the students can do in order to test some of these findings so that we can replicate their work.
m. Follow an ELISA protocol, record the procedure and results in their legal scientific notebook, and analyze the results to make valid conclusions.
n. Use Excel to make a standard curve to use as a reference to determine their antibody concentrations of their unknown samples.
o. Graph their standard curve data on a standard sheet of logarithmic graph paper to use as a reference to determine their antibody concentrations in their unknown samples.
p. Calculate the averages of their replicates to get a more valid value of absorbance before using it to determine their concentration using the standard curve.

q. Determine next steps in the study.
r. Analyze and record data based on cell size and granularity on forward vs. side scatter plots in flow cytometry.
s. Analyze and record data from samples stained with fluorochromes in 2 parameter dot plots and 1 parameter histograms.
t. Diagnose a patient with a given disorder based on given patient values, reference ranges of healthy individuals, and disease phenotype.
u. Use sterile technique to properly inoculate bacteria safely and without contamination.
v. Analyze results from antibiotic resistance assays accurately by measuring zones of inhibition.
w. Draw conclusions about effectiveness of antibiotic based on antibiotic resistance assay results.
x. Design and conduct their own experiment to test if their patient has an allergy to newly identified antibiotic that was found to work against the MRSA outbreak in the Antibiotic Resistance Assay.
y. Draw accurate conclusions about test results.

IV. Time Requirements:

This unit lasts about 29 days of instructing utilizing 42 minute class periods. Longer class periods would help expedite some lab activities and shorten the number of days required.

V. Advanced Preparations:

a. Introduction to Immunology Part 1 and Cell Research Project

- Determine if you will make this an individual project or group project based on class size.
- Cut out list of immune cells and have them folded in a bucket for students to draw from OR have cells preassigned to students based on ability and difficulty of information.
- Copy rubric for yourself and the students.
- Copy checklist for students.
- Find and print reference immune cell interaction diagram to reference as students collaboratively makes theirs on the board.

b. Introduction to Immunology Part 2 and Vaccines Article

- Choose articles and make copies for your groups.
  NOTE: ALL ARTICLES BELOW ARE SUMMARIES OF STUDIES. THE ACTUAL RESEARCH ARTICLES THEY CITE FROM ARE AT LINKS THAT CAN BE FOUND AT THE BOTTOM OF EACH WEBPAGE. This was to have both levels of articles for diverse learners. You can also choose any article.

i. "Safeguarding Our Health: Vaccines Protect Us All." *NIH News in Health*. NIH, July 2016. Web. 19 July 2016. http://newsinhealth.nih.gov/issue/Jul2016/Feature1

ii. "Finding Factors That Protect against Flu." *NIH Research Matters*. Ed. Harrison Wein. National Institutes of Health (NIH), 26 Apr. 2016. Web. 19 July 2016. http://www.nih.gov/news-events/nih-research-matters/finding-factors-protect-against-flu

iii. "Progress Toward an H7N9 Avian Flu Vaccine." *NIH Research Matters*. Ed. Harrison Wein. National Institute of Health (NIH), 20 Oct. 2016. Web. 19 July 2016. https://www.nih.gov/news-events/nih-research-matters/progress-toward-h7n9-avian-flu-vaccine

iv. Routh, Jennifer. "Novel Strategy May Improve Seasonal Flu Vaccine Effectiveness." *National Institute of Allergy and Infectious Disease*. N.p., 23 May 2016. Web. 19 July 2016. www.niaid.nih.gov/news/newsreleases/2016/pages/seasnal-flu-vaccine-strategy.asx

v. "Strategy May Improve Seasonal Flu Vaccines." *NIH Research Matters*. Ed. Harrison Wein. National Institute of Health (NIH), 14 June 2016. Web. 19 July 2016. https://www.nih.gov/news-events/nih-research-matters/strategy-may-improve-seasonal-flu-vaccines

vi. Wein, Harrison. "Antibodies Protect Against Range of Flu Viruses." *NIH Research Matters*. National Institute of Health (NIH), 27 Aug. 2012. Web. 19 July 2016. http://www.nih.gov/news-events/nih-research-matters/antibodies-protect-against-range-flu-viruses

vii. Wein, Harrison. "Learning What H5N1 Needs to Spread." *NIH Research Matters*. National Institute of Health (NIH), 9 July 2016. Web. 19 July 2016. http://www.nih.gov/news-events/nih-research-matters/learning-what-h5n1-needs-spread

iii. Quantitative ELISA Lab (see lab handout for specifics about reagents)

- Print ELISA PowerPoint out for students.
- Order ELISA Kit, 96 well plates, 20-200ul adjustable pipet, and a box of 20-200ul pipet tips.- see Materials and Equipment section below.
- Rehydrate antibodies and antigen to 50x concentration.
- Make 1x solutions of antibodies, antigen, PBS and other reagents in kit.
- Setup student work stations the day before to maximize class time for the lab.
- Share Excel spreadsheet with students via google sheets, e-mail, or upload to your class website.

v. Diagnosis of HIV/AIDS with the use of Flow Cytometry

- Preview Power Point Presentation and make any desired changes.
- Print patient serum sample sheets.
- Cut out individual cells from patient serum samples and place in Ziploc bag.
- Label front of Ziploc bag with patient information on each sheet.
- Print student handouts- 1 per group (3 students per group).

v. Antibiotic Resistance Assay

- 1 day before the lab:

a. If your *E. coli* plate that was ordered from Flinn Scientific has lawn growth, you will have to prepare another stock plate to isolate a single colony of E. coli in order to start your broth culture on the first official day of the experiment. To do this you will use sterile technique to pour 20 ml's of LB agar into a sterile 100mm petri dish, flame sterilize an inoculating loop, let loop cool to room temperature, pick up a small amount of E. coli from Flinn Scientific E. coli plate and triple streak it on the fresh LB agar. In order to use sterile technique without a laminar flow hood, you should sterilize your work surfaces with 10% bleach and 70% ethanol. You should also have a Bunsen burner nearby for flame sterilization of your inoculating loop and to prevent air contaminants from falling on your plate. You should also be careful not to set the petri dish lid or inoculating loop on any surface that is not sterile.
b. You triple streak your plate in order to create individual colonies.

Individual colonies are said to descend from a single E. coli cell, therefore all bacteria in that colony should be genetically identical. To triple streak a plate you will divide the plate into 3 sections by drawing a "T" on the bottom of the petri dish, like shown below. You will flame sterilize your loop, allow it to cool, pick up a small amount of bacteria from your ordered plate of E. coli, and streak it in a zigzag fashion in the area labeled #1. You will then flame sterilize your loop again, allow it to cool, and do a single drag with the inoculating loop through area #1 and drag it into area #2. Zigzag the loop without crossing over into area #1 or #3 anymore. Finally, flame sterilize the loop a third time, allow to cool, and do a single drag with the inoculating loop through area #2 into area #3. Zigzag the loop without crossing back over into areas #1 or #2. Close the plate lid and incubate at 37□C overnight upside down to prevent condensation formation on the agars surface.

vi. Ouchterlony Assay Development

- Order all lab materials listed.
- Make the 6 patient serum samples in 6 different 1.5 ml Eppendorf tubes (or any other tube that can be sealed). You are mixing the antibodies the serum samples because the patients serum will contain the antibodies against the antibiotics.
    a. Patient A= 20 µl goat anti-horse serum + 50 µl 1xPBS
    b. Patient B= 20 µul goat anti-swine serum + 50 µl 1xPBS
    c. Patient C= 20 µl goat anti-bovine serum + 50 µl 1xPBS
    d. Patient D= 20 µl goat anti-horse serum + 20 µl goat anti-swine serum + 30 µl 1xPBS
    e. Patient E= 20 µl goat anti-horse serum + 20 µl goat anti-bovine serum + 30 µl 1xPBS
    f. Patient F= 20 µl goat anti-swine serum + 20 µl goat anti-bovine serum + 30 µl 1xPBS
- Make aliquots in 1.5 ml Eppendorf tubes for each group of the 3 antigen bottles and a negative control. Instead of the name of the serum, you will label them with the antibiotics in the Antibiotic Resistance Assay that produced the 3 best zones of inhibition (students should determine which antibiotics they want to test in the Ouchterlony assay first before you show them the possibilities, and you should ensure that you know which antigen corresponds to their new labels).

a. Antibiotic 1= 20 µl swine serum + 50 µl 1xPBS
b. Antibiotic 2= 20 µl horse serum + 50 µl 1xPBS
c. Antibiotic 3= 20 µl bovine serum + 50 µl 1xPBS
d. Negative control = 70 µl 1xPBS

- Print out student guide and rubric.
- Make sterile agarose gel and pre-pour plates for students (but not until after they give you final values). - Do not make wells in the agar for them since each student design might be different.

vii. Immune System and Assays Summative Assessment

- Print copies of the test for each student.

VI. Materials and Equipment:

iv. Introduction to Immunology Part 1 and Cell Research Project

- List of immune cells that can be assigned
- Computer with PowerPoint and internet connection
- Projector in class for presentations
- White Board and Dry Erase Markers

v. Introduction to Immunology Part 2 and Vaccines Article

- Markers – 1 or more per group
- Poster board- 1 per group
- Copies of articles and student guide worksheet

vi. Quantitative ELISA Lab

- ELISA Kit number 1: Bio-Rad: ELISA Immuno Explorer Kit Cat # 1662400EDU, Cost: $138.00 https://www.bio-rad.com/en-us/product/elisa-immuno-explorer-kit?tab=Ordering
- STORAGE: Parts of this kit need to be stored at 4°C and RT.
- SAFETY: Although nothing in this lab is toxic or infectious, students should wear gloves and goggles as a precaution. If any substance gets in the eyes, they should flush their eyes for 15 min with water. Students should wash their hands before exiting the lab.
- NOTES: You can only do 5 groups with 1 kit for the protocol I have made to go with this lesson. I strongly recommend this kit if you have the time to go through it. I wrote my own protocol to this kit that is attached which fits in with the lesson on vaccinations. But if you don't like my protocol, this kit offers 3 different protocols as well as a way to quantify your results. It offers plenty of background knowledge and information to guide you through the

lab. It also offers a Zombie Apocalypse rendition of disease spread to interest the students. You can find these resources at the link above for ordering the product.

- 5x 96- well plates (some strips of wells come with the kit, but for the modified protocol, you need an entire plate per group)*-can be ordered from multiple suppliers.
- 5x 20-200 µl adjustable micropipette box-can be ordered from multiple suppliers.
- 5x boxes of micropipette tips to fit micropipette- micropipette dependent.
- 5- 200 ml beakers
- 10x large stacks of paper towels
- 5x black Sharpies
- 15x 5 ml test tubes with rubber stoppers or parafilm*
- 5x test tube racks*
- 5x- 1.5 ml tube racks
- Aluminum foil*

**** Extra needs off basic protocol if you are doing my modified protocol.***

iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

- Power point
- Projector and sound to show video
- Attached Student handouts
- Calculator
- Printed and cut out serum samples in Ziploc bag

v. Antibiotic Resistance Assay

- Antibiotic Disk Mini Set: Carolina Biological, Item # 806499, $51.95
- Antibiotic Sensitivity Disks, Blank, Sterile, Vial 50: Carolina Biological, Item #805091, $11.50
- Antibiotic Disk Dispenser: Carolina Biological, Item #806490, $15.95 (6 needed)
- Luria Broth Agar Ready to Pour Media Set: Carolina Biological, Item #216620, $27.75
  *You can get this cheaper if you buy the powder and make yourself
- Luria Broth, 50-ml bottle, 5-pack, sterile: Carolina Biological, Item

#216650, $24.00
*You can get this cheaper if you buy the powder and make yourself

- Bacterial Culture, Escherichia coli: Flinn Scientific, Item # LM1006. $11.10
- Inoculating loops, Nichrome Wire: Flinn Scientific, Item #AP1051, $2.75 each (6 pk) **OR**
- Glass Plate spreader: Carolina Biological, Item #703413, $6.25 (x6)
- Sterile transfer pipets, pack of 100: Carolina Biological, Item #214551, $33.00
- 100 mm x 15 mm petri dishes, sterile- 20 pack: Carolina Biological, Item #741250, $5.95 (x2)
- 10% bleach solution
- 70% Ethanol solution
- Bunsen Burners
- Paper towels
- Permanent marker
- Incubator (optional)- If you don't have an incubator, you can increase all incubation times to 48 hours and incubate at room temperature.

vii. Ouchterlony Assay Development

- Serum Antibody Set: Carolina Biological, Item #202102, $112.00
- Serum Antigen Set: Carolina Biological, Item #202101, $64.75
- 0.9% Agarose is PBS with 8% Sodium chloride
  *You can get this cheaper if you buy the powder and make yourself
- Sterile transfer pipets, pack of 100: Carolina Biological, Item #214551, $33.00
- 35mm x15mm petri dishes, sterile, pack of 20: Carolina Biological, Item #741246, $6.35 (x2)
- Phosphate Buffered Saline Solution-500 ml: Ward's Science, Item #470302-018, $13.95
  *You can get this cheaper if you buy the reagents and make it yourself.
- 1.5 ml Eppendorf tubes (x30) or any other sealable tubes.
- Permanent marker
- Gloves
- Goggles

VII. Students Prior Knowledge

vii. Introduction to Immunology Part 1 and Cell Research Project

- The teacher should review slides: 1-9, 12-20, 25-29 from the Introduction to Immunology PowerPoint attached in detail while skimming the slides in between. This will give the students the prior knowledge about the difference between innate and adaptive immunity as well as basic information on WBC formation and locations in the body.
- Students should know how to complete internet searches.
- Students should know how to make a presentation in Microsoft PowerPoint.
- Students should know how to properly cite sources. owl.english.purdue.edu and www.easybib.com are useful tools if they are struggling.

viii. Introduction to Immunology Part 2 and Vaccines Article

- Types of immune cells and their role in the immune system
- A general understanding of how vaccination works

ix. Quantitative ELISA Lab

- Students should have a general understanding of antibodies, their specificity, their binding habits, and their location in the body.
- Students should know what an enzyme is and how it works.
- Students should know how to use pipettes and accurately read volumes.
- Students should know what ELISA stands for, what it tests for, and the general procedure of how it works before starting the lab.

x. Diagnosis of HIV/AIDS with the use of Flow Cytometry

- Basic knowledge of the immune system
- Branches of immune system
- WBCs and their general functions in the immune system
- Cell surface receptors and antibodies
- Basic knowledge of viruses and other pathogens.
- Basic knowledge of electron excitation.

xi. Antibiotic Resistance

- Types of mutations and how they can affect protein structure and function
- Gene transfer
- Plasmids

- Basic understanding of antibiotics and antimicrobial agents

xii. Ouchterlony Assay Development

- required parts of an experiment to be successful, unbiased, and reproducible
- antibody specificity
- allergic response
- antigens

xiii. Immune System and Assays Summative Assessment

- All knowledge acquired in this unit will be assessed.

VIII. Assessment Methods

i. Introduction to Immunology Part 1 and Cell Research Project

- Give students checklist and rubric to guide the structure of their presentation.
- Use attached rubric to grade presentation.
- Visually check the class diagram of cell interaction and guide mistakes by asking probing questions. (Examples: Is that cell supposed to be in the innate or adaptive branch of the immune system? How does that cell interact with the other cells in the same system?)

ii. Introduction to Immunology Part 2 and Vaccines Article

- Attached Vaccination Article Review Activity Rubric
- Class brainstorm experimental activity.

iii. Quantitative ELISA Lab

- Use attached rubric to grade lab notebook which should include the following information:
    a. pre-lab questions and answers
    b. Procedure written in step by step, coherent form.
    c. Data recoded in appropriate tables with labels
    d. Graphs
    e. Post lab question and answers
    f. Written conclusion

iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

- Grade attached student worksheets for completion and accuracy. Students only get points for each question or graph that have correct answers.

v. Antibiotic Resistance Assay

- Lab notebook pre-lab, procedure, post-lab and conclusion with rubric

vi. Ouchterlony Assay Development

- Their lab protocol will be assessed for accuracy and completion with Ouchterlony Lab Protocol Rubric.
- Their lab results and conclusions will be assessed for accuracy and completion with the Ouchterlony Lab Notebook/Results/Conclusion Rubric

vii. Immune System and Assays Summative Assessment

- Grades will be taken according to directions outlined on test.

IX. Daily Unit Plans:

i. Day 1:

- First 5-10 students will create a KWL about the immune system in their notes and discuss.
- Remainder of time show students the attached video links (37:50 min total):

a. Lymphatic System: Crash Course A&P #44 (9:19): https://www.youtube.com/watch?v=I7orwMgTQ5I

b. Immune System, Part 1: Crash Course A&P #45 (9:12): https://www.youtube.com/watch?v=GIJK3dwCWCw

c. Immune System, Part 2: Crash Course A&P #46 (9:43): https://www.youtube.com/watch?v=2DFN4IBZ3rI

d. Immune System, Part 3: Crash Course A&P #47 (9:36): https://www.youtube.com/watch?v=rd2cf5hValM

ii. Day 2:

- Pass out Introduction to Immunology PowerPoint to students.
- Go over slides 1-9, 12-20, 25-29 in detail while skimming over the slides in between because they will complete a research project on the specific immune cells the next day.

i. Day 3: Move onto Immune Cell Research Project to see specifics.

- Pass out and discuss project checklist, assign cells, and start research.

Immune Cell Research Project Rubric Name _____

Topic	1	2	3
Name of Cell	Name of cell is present	N/A	N/A
% WBC	Percentage of total blood cells given	Percentage of WBC given.	N/A
Pathway of formation (X2)	Only steps of formation given	Steps of formation and maturation site given	Steps of formation, maturation site, and factors all included.
Main function (x2)	Little understanding of function, missing basic key concepts	Full understanding of basic function, all main concepts covered.	N/A
Innate vs. Adaptive (x2)	Student places it in the correct category, but does not justify their selection with innate or adaptive qualities.	Student places it in the correct category(s), and justifies their selection with innate or adaptive system qualities.	N/A
Surface markers	Student includes surface markers that are not distinct to that cell type.	Student includes only 1 surface marker that is distinct to that cell type.	Student includes multiple surface markers that are distinct to their cell type.
Interactions (x3)	Student only includes how the cell interacts with pathogens	Student includes how cells interact with pathogens and other cells, but without specifics of types of receptors/cytokines.	Student includes how cells interact with pathogens and other cells with specifics of receptor names and cytokines.
Picture of cell	Picture of cell	Picture of cell with citation	N/A
Diagram of interactions	Diagram of cell interactions	Diagram of cell interactions with citations	N/A
Diagram of cell development	Diagram of cell development	Diagram of cell development with citations	N/A
Presentation (x2)	Presentation is of poor quality and is not complete AND/OR Student is not looking at audience and reading off board.	Presentation is of decent quality, AND/OR student is looking at the board frequently with little eye contact with audience.	Presentation is high quality, and student is looking at the audience most of the time, just looking at presentation for ques and major facts.
Citations (x2)	Citations are included, but not in correct MLA or APA format.	Citations for cell information are included and in correct MLA or APA format.	N/A
Checkpoint questions	One checkpoint question included	Two checkpoint questions are included	Three checkpoint questions are included
Total			_____/48

Immune Cell Research Project Cell List

The list below is a list of some of the most common white blood cells in the human body. Each student should get one of these cells and complete the research project by answering all of the questions on the checklist above. You can pick and choose from this list based on class size. You can also pair students up on the project based on class size as well. If you want additional groups, you can add subsets of cells to the list such as Th1, Th2, Th17, Treg (all types of Helper T-cells) as well as subsets from other types of cells to differentiate.

Plasma B-cell
Memory B-cell
Cytotoxic T-cell (CD8 T-cells)
Helper T-cell (CD4 T-cells)
Macrophage
Dendritic cell
Natural Killer Cell
Natural Killer T cell
Basophil
Eosinophil
Neutrophil
Mast Cell

Immune Cell Research Project Presentation Criteria Checklist

Name_____

Assigned WBC: _____

_____1. Name of cell (1 pt)
_____2. Percentage of WBC population (2 pts)
_____3. Pathway of formation from hematopoietic stem cell. (6 pts)
   a. Are there multiple steps in this formation (if so, what are they?)
   b. Do they need to go somewhere else to finally mature? If so where?
_____4. What is its main function of this cell in the immune system? (4 pts)
_____5. Is this cell part of the innate or adaptive immunity system? Or does it help cross the bridge between the two systems? Justify your answer. (4 pts)
_____6. What distinct surface markers does the cell have? (3 pts)
_____7. How does this cell interact with other cells and pathogens? (9 pts)
   a. If direct recognition, how does it alert other cells in the immune system?
   b. If it needs to be presented to, what cells do the presenting?
_____8. Picture of your cell with citations (2 pts)
_____9. Diagram of cell development citations (2 pts)
_____10. Diagram of interactions with pathogens or other immune cells with citations (2 pts)
_____11. At least 3 check point questions to ensure student understanding (3pts)
_____12. Presentation (6 pts)
_____13. Citations for information (MLA or APA) (4 pts)

TOTAL : _____/48 points

iv. Day 4: Continue Research and presentation preparation.

v. Day 5: Continue Research and presentation preparation (Note: You can remove this day if you assign students to complete work outside of class.)

vi. Day 6: Cell Presentations (Note: You may need to take an extra day for presentations depending on class size and number of groups.)

vii. Day 7:

- ½ class period (20 min) to review slides 10-11 and 21-24 in Introduction to Immunology PowerPoint presentation to ensure accurate understanding of all immune cells.

viii. Day 8: Finish Introduction to Immunology PowerPoint, slides 30-40 and watch videos embedded in PowerPoint.

- Will We Ever Cure HIV by DNews (3:47 min): www.youtube.com/watch?v=E2Hfz45e17w
- Rise of the Superbug: Antibiotic Resistant Bacteria: Dr. Karl Klose at TEDx SanAntonio (11:03 min): https://www.youtube.com/watch?v=ikZQPB45Zbw

ix. Day 9: Vaccination research article assignment

- Assign one research article to each lab group.
- Pass out Vaccination Article Review Activity Student Guide and rubric.
- Pass out poster board and markers.
- Students should write required information on posters to present and discuss with class.

Vaccination Article Review Activity Rubric Name _____

Topic	2	4
Title	Title present	NA
Journal	Journal title present	NA
Date Published	Published date present	NA
Author(s)	Authors listed	NA
Important vocabulary	Key terms listed, but not defined	Key terms listed and defined
Treatment groups	Only some treatment groups identified	All treatment groups identified
Procedure (x2)	Steps in procedure are missing, incomplete, or mixed up to the point that the procedure is unclear	Steps in procedure are presented in order and none are missing.
Data	Data is discussed, but some key data is missing OR does not tie the data to the conclusion.	Key data is identified and discussed and explained why it is important to their conclusions.
Conclusion (x2)	Conclusion is given, but missing key aspects.	Conclusion is fully explained highlighting all aspects.
Class discussion	Class discussion was not focused on the topic of vaccinations.	Class discussion was focused and generated good feedback.
Total		_____/40

x. Day 10: Vaccination Article Review Activity Presentations
    - Students present and lead class discussions on their articles data and conclusions.
    - Students will brainstorm as a class to create experiments that will test the papers' proposals. Teacher will guide their experimental setup ideas by making sure that the students think about controls, time frame, number of participants, etc. to make the experiment valid. Teacher will write their ideas on the board as they talk through it.

xi. Day 11: ELISA Introduction
    - ELISA PowerPoint Notes
    - ELISA Video of Quick overview:
      Enzyme-Linked Immunosorbent Assay (ELISA) by openmichigan (1:48 min) quick overview:
      https://www.youtube.com/watch?v=RRbuz3VQ100

xii. Day 12: ELISA Pre-Lab
    - ELISA Kit Instructional Video:
      Quantitative ELISA by BioRadLifeScience (12:17 min) the video that goes over the ELISA kit used in class:
      https://www.youtube.com/watch?v=849HN1ueUhs
    - Pass out ELISA Lab Packet and rubric.
    - Discuss background, materials, and assign Pre-lab for homework.

ELISA Assay Lab Notebook Rubric

Topic	2	4	6
Title and page number	Title OR page numbers present	Both title and page numbers present	NA
Purpose	Purpose is included but incorrect	Purpose is included and correct	NA
Pre-lab questions	Prelab questions 1/3 complete or are very inaccurate	Pre-lab questions 2/3 complete or are somewhat inaccurate	Pre-lab questions fully completed and accurate
Procedure (x4)	Procedure is incomplete and does not have clear numbered steps	Procedure is incomplete, but has clearly numbered steps	Procedure is clearly numbered and complete
Data collection and recording (x8)	There are no data tables and incomplete/incorrect data is recorded	There are no data tables, but data is accurate and complete OR vice versa	There are complete and accurate data tables
Graphs (x3)	Both graphs are incomplete, incorrect, or only one graph is completed while the other is missing	Both graphs are completed, but there are some errors.	Both graphs are completed and accurate with axis labels and graph titles present
Post lab questions	Post lab questions are 1/3 complete or very inaccurate	Post lab questions are 2/3 complete or have some inaccuracies	Post lab questions are complete and accurate
Conclusion(x2)	Conclusion is inaccurate or incomplete, missing 2 or more of the 4 parts: RE, PE, PA, and next steps	Conclusions is accurate but incomplete and is missing 1 of the 4 parts: RE, PE, PA, and next steps	Conclusion is accurate and complete with all 4 parts present.
Total			122

Quantitative ELISA Laboratory Exercise to Test Immune Response to Various Vaccination Methods- Teacher Booklet

This protocol has been modified from the protocol in the Bio-Rad: ELISA Immuno Explorer Kit Cat # 1662400EDU.

This protocol simulates a diagnostic blood test for the detection of serum antibodies. With this protocol, students perform an ELISA to detect circulating antibodies in the blood as an indication of exposure to a disease-causing agent. Each student is provided with a simulated serum sample and asked to assay the sample for the presence of antibodies.

You may choose to focus on any of a variety of diseases such as HIV, West Nile virus (in people or animals), SARS, Lyme disease, trichinosis, tuberculosis, or any other disease that may stimulate your students' interest. This technique is useful for detection and diagnosis "post-infection" where the antigen itself is undetectable in the body. Once the body has mounted an immune response, antibodies are present in the blood serum and can be detected. For example, until very recently it was not possible to use ELISA to detect the HIV virus directly, but serum antibodies against the virus could be detected in vitro using ELISA. Hence, the ELISA antibody test was the only rapid way to diagnose HIV infection. Today, as the result of extensive investment into research on the biology of HIV/AIDS, it is possible to detect the HIV antigen in the blood directly by ELISA, allowing early treatment and extension of life.

To create a relevant and meaningful classroom context for this activity, the introductory pages to this manual as well as Appendices A and B provide background vocabulary and factual and conceptual lecture points. Appendix C contains useful information about specific diseases and classroom presentation scenarios for this ELISA antibody test protocol. The assay can be personalized with students assaying their "own" simulated serum samples, or it can be performed as a role-playing activity where students assay patient samples in a clinical lab at a local hospital.

While ELISA gives a definitive qualitative (yes/no) answer, a major strength lies in that it can also give quantitative (how much?) information. This lesson also provides a protocol that adapts this assay for use as a quantitative ELISA to assay either antigen or antibody levels. The following protocol is written to quantitate levels of antibody in serum with protocol III as a basis, but it can easily be adapted to quantitate levels of antigen with protocol II as a basis. It can be adapted for protocol I, but the results will probably not be in the linear range and therefore will not be very accurate.

This extension activity uses serial dilution to generate dilutions of known antibody concentration. As the concentration of antigen or antibody increases, so does the intensity of the blue color in the wells. The blue color absorbs light at a specific wavelength, and this absorbance can be measured with a microplate reader. Students compare their test samples to

the dilution series to calculate (or estimate) the concentration of the test samples. Using the ENZO ELISA plate reader application from the Google Play store on their phone, students can snap a picture of their ELISA plate and read the absorbance values of their wells at 595 nm, generate a standard curve in excel or on logarithmic graph paper, and calculate the concentration of their samples. If no microplate reader is available, the students can visually match the intensity of their samples with the samples in their dilution series and estimate the concentration of antigen in their samples.

Implementation Timeline

Day 1	Lecture Discussion on ELISA
Day 2	Pre-lab
Day 3	ELISA Lab
Day 4	ELISA data analysis

Quantitative ELISA: Step-by-Step Instructor's Advance Preparation Guide

We strongly recommend that you familiarize yourself with the basic protocols in this kit prior to performing this lab. It is especially important to read the information and notes in the tables within this section concerning reagent stability.

These instructions are for the setup of 5 student workstations of 4 students each.

Objectives:

1. Prepare buffers
2. Rehydrate the freeze-dried antigen, primary antibody. And secondary antibody to make 50 x stocks
3. Dilute 50 x stock solutions to 1x working solutions
4. Dispense reagents for student workstations
5. Set out student workstations

Time Required: 1-3 hours

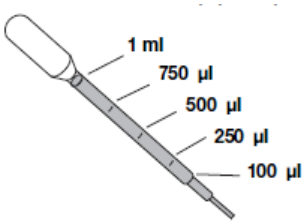
Preparation Timeframe

We recommend rehydrating and diluting the antigen and primary antibody no more than 3 days before the lesson, and the secondary antibody less than 24 hours before the lesson. We also suggest using sterile, distilled water to prepare the 1x PBS to avoid contaminating rehydrated reagents. These reagents must be kept on ice or in the refrigerator if prepared more than 4 hours before the lesson.

Note: If you are planning to use this kit for multiple lab sessions over a 1- or 2-week period, we strongly suggest using sterile water to prepare the PBS avoid contaminating reagents. (Water can be sterilized by boiling it in a microwave oven for 5 minutes in a loosely capped bottle; after you remove the bottle from the microwave oven, let it cool, then secure the cap.) Dilute only as much concentrated antibody and antigen as required for each lab session. The rehydrated antibodies are 50x concentrates. Store the remaining concentrated antigen and antibodies in the refrigerator at 4°C. We do not recommend storing the concentrated antibody and antigen for more than 2 weeks, even at 4°C. Do not freeze the solutions.

Volume Measurements

This kit contains graduated disposable plastic transfer pipets (DPTPs) to use for preparing some of the reagents where volumes between 250 microliters (µl) and 5 milliliters (ml) are required. In addition, adjustable- or fixed-volume micropipettes are needed to measure 50 µl volumes. The illustration shows the marks on the DPTP corresponding to the volumes to be measured. Volumes over 1 ml will require multiple additions. For each step of the laboratory preparation use a fresh DPTP or a fresh pipet tip.



Measuring liquids that contain detergents that foam (e.g., the wash buffer) requires that you read the volume at the interface of the liquid and the bubbles.

Supplied Reagents	**Quantity**
Antigen (chicken gamma globulin), freeze-dried	1 vial
Primary antibody (rabbit anti-chicken polyclonal antibody), freeze-dried	1 vial
Secondary antibody (goat anti-rabbit antibody conjugated to horseradish peroxidase(HRP)), freeze-dried	1 vial
HRP enzyme substrate (TMB)	1 bottle
10x phosphate buffered saline (PBS)	1 bottle
10% Tween 20	1 bottle

Required Reagents

Distilled water, sterile is recommended, 1 L

Step 1. Prepare buffers.

We recommend you use a 100 ml and a 1 liter graduated cylinder for preparing the buffer solutions. You will also need 1 liter of distilled water.

Buffer	Volume	Reagent	Used for
1x PBS, 100 ml	90 ml	Distilled water	•Rehydrating antigen, primary and secondary antibodies to make 50x reagent stock solutions • Diluting 50x antigen
	10 ml	10x PBS	
Wash buffer, 900 ml	805 ml	Distilled water	•Dilution of 50x primary antibody stock for positive control and positive student samples •negative controls •negative student serum samples •dilution of 50x antibody stock • Plate Washing
	90 ml	10x PBS	
	4.5 ml	10% Tween 20	

Step 2. Rehydrate the freeze-dried antigen, primary antibody, and secondary antibody.

Carefully remove the stoppers from the three freeze-dried reagents and use a fresh pipet to add 0.5 ml 1x PBS to each. Close the stoppers and shake to mix. These solutions are 50x concentrates, or stock solutions. NOTE: You must not use wash buffer in this step.

Freeze-Dried Reagent	Protocol for 50x Stock Solution	Used for
Antigen	Add 0.5 ml of 1x PBS to vial	• Purified antigen
Primary antibody	Add 0.5 ml of 1x PBS to vial	• Positive controls • Positive student serum samples

Secondary antibody	Add 0.5 ml of 1x PBS to vial	• Secondary antibody

Step 3: Dilute 50 x stock reagents.

Label one 50 ml tube each for 1x antigen, 1x primary antibody and 1x secondary antibody. Use a fresh DPTP to add the contents of the appropriate stock to the corresponding 50 ml tube.

Diluted Solution	Volume Reagent	Used for
1x Antigen, label one 50 ml tubes	24.5 ml 1x PBS	• purified antigen
	0.5 ml 50x antigen stock	
	NOTE: you must not add any buffer containing Tween 20 to the antigen, or the experiment will not work. • Use the DPTP to rinse out the vial with some of the diluted reagent to ensure that all of the stock solution is used. • Close the cap and shake to mix.	
1x serum (1x Primary antibody), label one 50 ml tube	24.5 ml Wash buffer	•positive controls • positive student serum samples
	0.5 ml 50 x primary antibody stock	
	• Use the DPTP to rinse out the vial with some of the diluted reagent to ensure that all of the stock solution is used. • Close the cap and shake to mix.	
1x secondary antibody, label one 50 ml tube	24.5ml Wash Buffer	•Secondary antibody
	0.5 ml 50x secondary antibody stock	
	•Dilute the secondary antibody less than 24 hours before the start of the lesson. •Use the DTP to rinse the vial with some of the diluted reagent to ensure that all the stock solution is used. •close cap and shake to mix.	

Step 4: Dispense reagents for student workstations.

Tubes	Description	Label	Content (Each Tube)
Violet tubes, 5	Positive controls	"+"	350 µl 1x serum (1x primary antibody)
Blue tubes, 5	Negative controls	"-"	350 µl wash buffer
5 ml tubes labeled "AG", 5	Purified antigen	"AG"	5 ml 1x antigen
5 ml tubes labeled "SA"	Secondary Antibody	"SA"	5 ml 1x secondary antibody
5 ml tubes, 5	Enzyme substrate	"SUB"	5 ml HRP enzyme substrate (TMB)
	NOTE: TMB is light sensitive, so you need to keep these tubes in the dark by covering with foil or under a box.		
Yellow Tubes, 5	Standards	"STAN"	250 µl 1x primary anti2ody
Yellow Tubes, 5	Patient 1-negative control	"P1"	250 µl wash buffer
Yellow Tubes, 5	Patient 2- viral vaccine	"P2"	*250 µl of 3.9 ng/ml primary antibody (1:256 dilution of 1x stock)
Yellow Tubes, 5	Patient 3- viral booster	"P3"	*250 µl of 15.625 ng/ml primary antibody (1:64 dilution of 1x stock)
Yellow Tubes, 5	Patient 4-viral w/ low adjuvant	"P4"	*250 µl of 250 ng/ml primary antibody (1:4 dilution of 1x stock)
Yellow Tubes, 5	Patient 5- viral w/ high adjuvant	"P5"	*250 µl of 500 ng/ml primary antibody (1:2 dilution of 1x stock)

*To make the dilutions required for patient samples start with 1.5 ml of 1x primary antibody in an empty 5 ml tube. Label that tube "1:2". Add 1.5 ml of wash buffer to the tube. Fill 9 other empty 5 ml tubes with 1.5 ml of wash buffer. Label them "1:4", "1:8", "1:16", "1:32", "1:64", "1:128", and "1:256" respectively. Put all the tubes in order as listed. Take 1.5 ml from tube labeled "1:2" and add it to tube "1:4". Mix up and down. Take 1.5 ml from tube "1:4" and add to tube "1:8". Mix up and down. Continue this sequentially until you get to the last tube. You now have at least 1.5 ml's in all your required dilutions for your patient samples. Transfer 250 µl of each concentration of interest to the corresponding yellow patient tubes for each of your 5 groups. Ex. You will have 5 yellow- 1.5 ml tubes with 250 µl of 1:64 primary antibody each labeled "P3" for each group.

Step 5: Set out student workstations.

Student Workstation Checklist

One workstation serves 4 students.

Item (label)	Contents	Number	(✓)
Yellow tube (STAN)	standard (250 µl)	1 -1.5ml tube	☐
Yellow tube (P1)	Patient 1 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P2)	Patient 2 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P3)	Patient 3 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P4)	Patient 4 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P5)	Patient 5 (250 µl)	1 -1.5ml tube	☐
Violet tube (+)	positive control (350 µl)	1 -1.5ml tube	☐
Blue Tube (-)	negative control (350 µl)	1 -1.5ml tube	☐
Clear Test Tube* (AG)	Purified antigen (5 ml)	1 -**5ml** tube	☐
Clear Test Tube* (SA)	Secondary Antibody (5 ml)	1 -**5ml** tube	☐
Clear Test Tube ***covered in foil**** (SUB)	Enzyme Substrate (5 ml)	1 -**5ml** tube	☐
1 -96 well ELISA plate*		1	☐
50 µl fixed volume micropipette* OR 20-200 µl adjustable micropipette*		1	☐
Yellow Tips to fit micropipette*		1 box	☐
Disposable plastic transfer pipet		1	☐
120-150 ml wash buffer in 200 ml beaker	1x PBS with 0.05% Tween 20	1	☐
Large stack of paper towels*		2 stacks	☐
Black marking pen*		1	☐

**** Items with * are NOT included in kit.***

Stopping points: Although this procedure is designed to fit into a single lesson period, you may stop the laboratory activity by adding wash buffer to the microplate wells at any stage after the addition of antigen and prior to the addition of enzyme substrate. Place the microplate strips and all the reagents in the refrigerator at 4°C overnight.

Instructor's Answer Key and Discussion Points

Pre-Lab Focus Questions

1. **How does the immune system protect us from disease?**
   The immune system includes physical barriers, such as the skin and mucous membranes that prevent pathogens from entering the body, and cellular responses, such as circulating macrophages that respond to foreign invaders. Our acquired immune system mounts a specific antibody response when the body is exposed to a foreign invader, and our immune cells attack the invader.
2. **How do doctors use the immune response to protect you from disease?**
   Doctors use the immune response when we are vaccinated against diseases. Our immune system remembers the pathogens to which we have been exposed, and the next time we are exposed to the pathogens our immune system attacks them more quickly and efficiently. Doctors take advantage of this priming effect by exposing us to inactivated pathogens (killed or weakened organisms that cannot make us sick) so that if we are later exposed to the live pathogen, our body will mount a strong and immediate antibody response, reducing or eliminating the chance that it will make us sick.
3. **What is an example of a disease that attacks the human immune system?**
   Diseases that attack the immune system include autoimmune diseases (e.g., rheumatoid arthritis, lupus, asthma, eczema, SCID) and AIDS. An extensive list can be found in Appendix A.
4. **What problems can prevent the immune system from working properly?**
   Problems with the immune system fall into three categories: hypersensitivity, immunodeficiency, and autoimmune diseases. Hypersensitivity occurs when the immune system overreacts to an antigen; hypersensitivity reactions include anaphylactic reactions, allergies, and contact sensitivity (e.g., reaction to poison ivy). Immunodeficiency means that an individual cannot mount an effective immune response. Immunodeficiency may be genetic (e.g., SCID or "bubble boy" disease) or induced by a disease (e.g., immunodeficiency from HIV infection) or by immunosuppressive drugs (e.g., drugs given after organ transplant to prevent rejection). Autoimmune disease results from the immune system inappropriately mounting an immune response to itself, (for example, diseases like lupus, rheumatoid arthritis, multiple sclerosis (MS), insulin-dependent diabetes, and celiac disease).
5. **Why is it important to be able to detect antibodies in people who don't appear sick?**
   It is important because people may be disease carriers even though they are not sick themselves. Typhoid fever is an example of a disease that has chronic carriers; up to 5% of individuals infected with typhoid fever excrete the bacteria for up to a year. For example, in the historical case of Typhoid Mary, Mary Mallon infected 47 people with typhoid fever over the course of 15 years, even though she was never ill herself.

   West Nile virus (WNV) is another good example of why it is important to detect

antibodies in people who aren't sick. WNV is spread by mosquitoes. Most people who are infected with West Nile have either no symptoms or very mild symptoms. To get a true picture of the epidemiology of West Nile virus, it is necessary to test for antibodies against WNV in many more individuals than just the few who develop symptoms. For example, if one person on a street becomes ill from WNV, testing all of his neighbors may show that dozens were infected with the virus but did not get sick. Information on infection rates, not just on rates of illness, is necessary to get an accurate picture of the disease. For a disease like HIV/AIDS, early detection of infection is crucial. People infected with HIV may have no symptoms at all for many years, yet if they begin treatment prior to the onset of disease, development of symptoms may be postponed indefinitely. In addition, during the asymptomatic phase of the infection, individuals infected with HIV and unaware of their infection could pass the virus on to other people with whom they have intimate contact.

6. **What does ELISA stand for?**
   Enzyme-linked immunosorbent assay.
7. **Why are enzymes used in this immunoassay?**
   Enzymes provide a way to see whether the primary antibody has attached to its target (antigen) in the microplate well. Primary and secondary antibodies are invisible, so a detection method is necessary. The enzyme horseradish peroxidase (HRP) is linked to the secondary antibody. HRP reacts with a colorless substrate in a chemical reaction that turns blue. If the secondary antibody is present in the well, the color change indicates a positive result.
8. **What is an adjuvant (you will need to look this up if you don't remember)?**
   An adjuvant is a pharm logical or immunological agent that improves the effects of vaccinations by increasing the immune response to induce a higher production of antibodies and memory cells.
9. **Predict which patient(s) you think will have the greatest immune response. How much greater do you think it will be from the other patients. Justify your answers.**
   Most students might predict patient 5 and with a 5-fold increase in immune response because it has 5 times greater the amount of adjuvant, but it is really only 2 times the response because the adjuvant has reached its plateau of effectiveness.
10. **Why do you need to assay positive and negative control samples as well as your experimental samples?**
    Controls are needed to make sure the experiment worked. If there are no positive controls and the sample is negative, we can't know if the sample was truly negative or if the assay didn't work. Conversely, without a negative control, there is no way of knowing
    if all samples (positive or not) would have given a positive result.
11. **If we have positive and negative controls on our ELISA plate, why would we need to have Patient 1 as a negative control also?**
    You want to have someone who has been injected with a blank sample as well to ensure that just the action of the injection itself is the thing that elicited the increase in immune

response.

Post-Lab Focus Questions

1. **Which patient samples had antibodies that were specific against the MMR disease?**
   Patients 2-5
2. **If they tested positive for antibodies, does this mean that they got the vaccination?**
   No, because there could have been a false positive due to experimental error.
3. **What if you ran this test in real life and Patient 1 came back positive for antibodies against MMR and you eliminated all technical errors like cross contamination, what would be one reason he could come back positive for antibodies against MMR?**
   They may have been exposed to MMR before the vaccination experiment. That is why we should assess patients for antibodies specific to the disease of interest before the trial as well. Experimental error OR cross contamination.
4. **Why did you assay your samples in duplicates?**
   To have greater accuracy and to eliminate error or outliers. We could have run in triplicates to be even more accurate and eliminate outlier data that could be due to human error, but there were not enough wells or reagents.
5. **When you added serum samples to the wells, what happened to the serum antibodies if the sample was positive? What if it was negative?**
   If the sample was positive, serum antibodies that recognize the purified antigen in the wells bound to the antigen. If the sample was negative, no antibodies bound.
6. **Why did you need to wash the wells after every step?**
   To ensure any unbound reagents are washed out of the wells to reduce experimental error and false positive results.
7. **When you added secondary antibody, what happened if your serum sample was positive? What if it was negative?**
   If it was positive, the secondary antibody bound to the antibodies that were present in the patient's serum. If it was negative the secondary antibodies were unable to bind and got washed away.
8. **What antibody-based tests can you buy at your local pharmacy?**
   Pregnancy tests and drug tests.
9. **Based on your data, which patient produced the greatest immune response? How do you know? Why do you think that was?**
   Patient 5 because it had the highest concentration of antibodies. I knew this because the wells were darker than any other patient AND the absorbance readings were greater than any other patient. When on the standard curve it gave an antibody concentration of about 500 ng/ml.
10. **Did that patient match your exact prediction? Was the concentration of antibody the same as your predicted value? If not, why do you think that was?**
    The patient was predicted correctly, but the concentration was less than I thought. I thought it would be 5 times as much as patient 4 because it had 5 times the amount of adjuvant, but the antibody response reached its plateau.

11. **Compare and contrast the accuracy of your eyeball sample concentrations to the actual concentrations obtained by both you hand calculating the absorbance of your samples and the excel calculations of the absorbance of your samples.**
    This is student dependent, but results should have been close with the computer graphing being the most accurate.

Laboratory Quick Guide
ELISA Antibody Test
Student Workstation Checklist

One workstation serves 4 students.

Item (label)	Contents	Number	(✓)
Yellow tube (STAN)	standard (250 µl)	1 -1.5ml tube	☐
Yellow tube (P1)	Patient 1 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P2)	Patient 2 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P3)	Patient 3 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P4)	Patient 4 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P5)	Patient 5 (250 µl)	1 -1.5ml tube	☐
Violet tube (+)	positive control (350 µl)	1 -1.5ml tube	☐
Blue Tube (-)	negative control (350 µl)	1 -1.5ml tube	☐
Clear Test Tube (AG)	Purified antigen (5 ml)	1 -**5ml** tube	☐
Clear Test Tube (SA)	Secondary Antibody (5 ml)	1 -**5ml** tube	☐
Clear Test Tube ***covered in foil*** (SUB)	Enzyme Substrate (5 ml)	1 -**5ml** tube	☐
1 -96 well ELISA plate		1	☐
50 µl fixed volume micropipette OR 20-200 µl adjustable micropipette		1	☐
Yellow Tips to fit micropipette		1 box	☐
Disposable plastic transfer pipet		1	☐
120-150 ml wash buffer in 200 ml beaker	1x PBS with 0.05% Tween 20	1	☐
Large stack of paper towels		2 stacks	☐
Black marking pen		1	☐

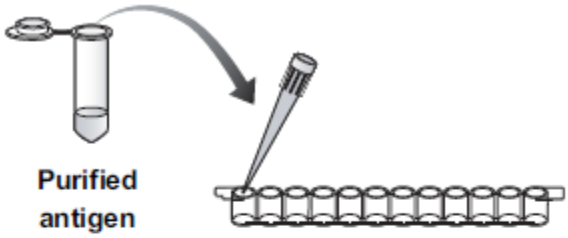
Protocol

1. Follow the 96 well ELISA Plate scheme provided below.

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	STAND 1000 ng	1:2 STAN	1:4 STAN	1:8 STAN	1:16 STAN	1:32 STAN	1:64 STAN	1:128 STAN	1:256 STAN	1:512 STAN	1:1024 STAN	1:2048 STAN
B	STAND 1000 ng	1:2 STAN	1:4 STAN	1:8 STAN	1:16 STAN	1:32 STAN	1:64 STAN	1:128 STAN	1:256 STAN	1:512 STAN	1:1024 STAN	1:2048 STAN
C	+	P1	P1	P2	P2	P3	P3	P4	P4	P5	P5	x
D	+	P1 1:2	P1 1:2	P2 1:2	P2 1:2	P3 1:2	P3 1:2	P4 1:2	P4 1:2	P5 1:2	P5 1:2	x
E	+	P1 1:4	P1 1:4	P2 1:4	P2 1:4	P3 1:4	P3 1:4	P4 1:4	P4 1:4	P5 1:4	P5 1:4	x
F	-	P1 1:8	P1 1:8	P2 1:8	P2 1:8	P3 1:8	P3 1:8	P4 1:8	P4 1:8	P5 1:8	P5 1:8	x
G	-	P1 1:16	P1 1:16	P2 1:16	P2 1:16	P3 1:16	P3 1:16	P4 1:16	P4 1:16	P5 1:16	P5 1:16	X
H	-	P1 1:32	P1 1:32	P 2 1:32	P2 1:32	P3 1:32	P3 1:32	P4 1:32	P4 1:32	P5 1:32	P5 1:32	x

2. Use a fresh pipet tip to transfer 50 µl of purified antigen (AG) into all wells EXCEPT Column 12 Rows C-H of the 96 well plate.

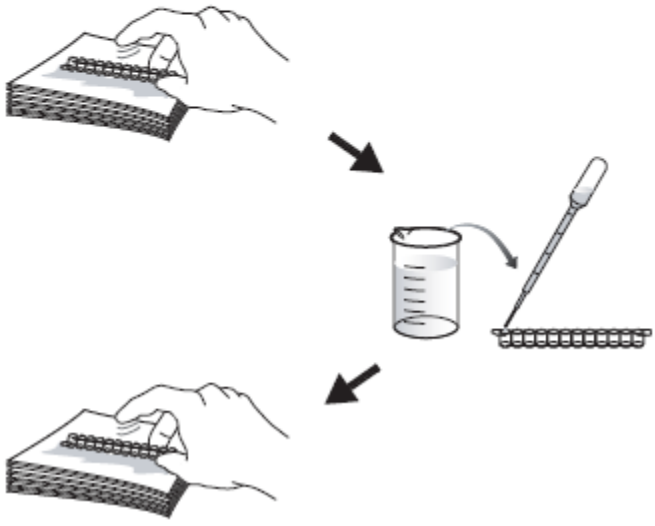


3. Wait 5 minutes for the antigen to bind to the plastic wells.

4. WASH:
   a. Tip the 96 well plate upside down onto the paper towels, and gently tap the plate a few times upside down. Make sure to avoid splashing sample back into wells.

   b. Discard the top paper towel.

   c. Use your transfer pipet to fill each



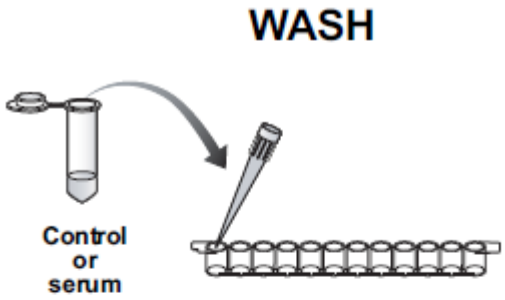
well with wash buffer, taking care not to spill over into neighboring wells. Note: the same transfer pipet is used for all washing steps.

d. Tip the microplate strip upside down onto the paper towels and tap.
e. Discard the top 2–3 paper towels.

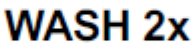
5. Repeat wash step 4.

6. Pipet 50 µl of wash buffer into each of your dilution wells before adding samples. Wells: A2-12, B2-12, D2-11, E2-11, F2-11, G2-11, H2-11.

7. Make your serial dilutions of your standard.
   a. Place 100 µl of your standard "STAN" into A1.
   b. Take 50 µl from A1 and place into A2.
   c. Pipet up and down to mix.
   d. Take 50 µl from A2 and place into A3.
   e. Pipet up and down to mix.
   f. Continue this until you get to A12.
   g. Get a fresh tip and repeat for row B.



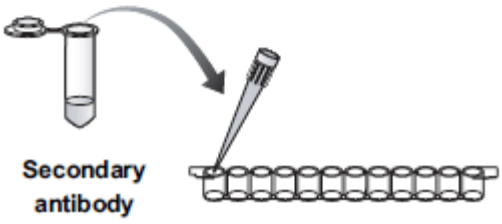
8. Make serial dilutions of your patient samples.
   a. Place 100 µl of your "P1" sample into C2.
   b. Take 50 µl of C2 and place into D2.
   c. Pipet up and down to mix.
   d. Take 50 µl of D2 and place into E2.
   e. Pipet up and down to mix.
   f. Continue this until you get to H2.
   g. Get fresh tip and repeat "P1" serial dilution in C3 to H3.



9. Get a fresh tip and repeat step 8 for all patient samples "P2" – "P5" in corresponding rows and columns in plate layout above.

10. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.

11. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.



12. Wait 5 minutes for the antibodies to bind to their targets.

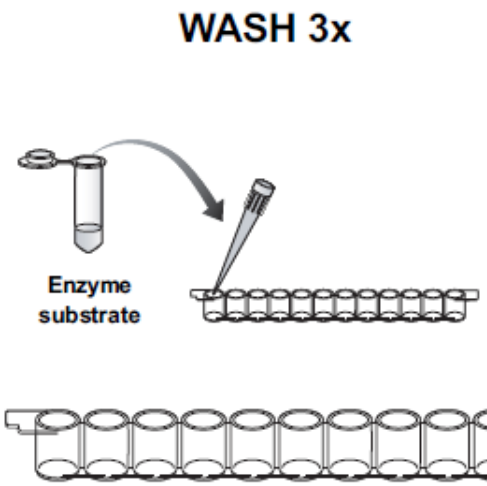
13. Wash the unbound primary antibody out of The wells by repeating all of wash step 4 **two** times.

14. Use a fresh pipet tip to transfer 50 µl of secondary antibody (SA) into all wells containing samples.

15. Wait 5 minutes for the antibodies to bind to their targets.

16. Wash the unbound secondary antibody out of the wells by repeating wash step 4 **three** times.

17. Use a fresh pipet tip to transfer 50 µl of enzyme substrate (SUB) into all wells containing samples.



18. Wait 5 minutes. Observe results and take picture with the ENZO ELISA Plate Reader application on your cell phone.
    Save picture for later analysis.

xiii. Day 13: ELISA Lab
- Students will complete the ELISA Lab during this class time.
- Students will take picture of completed ELISA plates with the ENZO plate reader app on their phone.

xiv. Day 14: ELISA data Analysis
- Students will read picture of their ELISA Plates in the ENZO ELISA plate reader app on their phone.
- Students will transfer absorbance readings to Microsoft excel document attached. Excel will automatically generate standard curve of data and figure out sample averages.
- Students will determine unknown sample concentrations using standard curve.
- Students will determine antibody titer of unknown samples.

xv. Day 15: ELISA data Analysis cont.
- Students will calculate and graph their standard curve by hand and compare their concentrations to concentrations obtained by excel.
- Students will answer post lab questions and write a valid, data based conclusion in their lab notebook.

xvi. Day 16: Flow Cytometry Power Point and Video

xvii. Day 17: Diagnosing HIV/AIDS with the use of Flow Cytometry Activity.
- Assign students a patient.
- Students sort “stained cells” in the serum sample.
- Students record data “HIV/AIDS Testing- Sample Transit, Receipt, and Report Form.”
- Students draw conclusions of diagnosis.

Name(s): _____

HIV/AIDS Testing-Sample Transit, Receipt, and Report Form

Directions: Complete the following form using the assigned patient serum sample in the given Ziploc bag**. Each paper cell represents 100 cells**. Use a calculator to compute percentages and ratios. (1 pt/blank= 40pts total)

A. Sample Transit and Receipt Form

Patient ID	Age	Sex	Date & time of Blood Collection	Signature of Phlebotomist	Receivers Signature at the testing Lab	Date of Blood sample receipt & time	Integrity of the blood sample on receipt

B. Sample Reporting Form

Name and Address of the Testing Lab: _____
Department of Testing Lab: _____
No. Assigned by the institute (you can make this up): _____
No. Assigned by the Clinic: _____
Referred by: _____
Date and time of Collection: _____

C. Results of Immunophenotyping:

Cell Type	**Raw number**	**% of all cells phenotyped**	**Reference ranges***
Stem cells			**1%**
Granulocytes: Neutrophils, eosinophils, and basophils			**41-79%**
Monocytes			**4-8%**
B-Lymphocytes			**1-7%**
Thrombocytes (platelets)			**4-7%**
T-lymphocytes (total)			**7-24%**
● Helper T-lymphocytes (CD4)			**4-20%**
● Cytotoxic T-lymphocytes (CD8)			**2-11%**

*Reference ranges were obtained from article: *Frequencies of Cell Types in Human Peripheral Blood*. STEMCELL Technologies, n.d. Web. 19 July 2016. <https://www.stemcell.com/media/files/wallchart/WA10006-Frequencies_Cell%20Types_Human_Peripheral_Blood.pdf>.

Parameter-T Cells	Value	Reference Ranges*	
		Male	Female
Absolute CD4 count		350-1440	450-1510
CD4 Percentage		30.4-61.6	34.9-62.1
Absolute CD8 count		200-1000	230-910
CD8 Percentage		15.9-45.8	16.7-40.6
CD4:CD8 ratio		0.7-3.4	1-3.3

HIV/AIDS specific results:

*Reference ranges were obtained from article: *Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men* by MK Maini, RJC Gilson, N Charvda, EJ Ross, AN Phillips, IVD Weller. Genitourin Med. 1996 Feb; 72(1): 27–31

Instrument Used: _____

Remarks/Diagnosis:_____

_____

_____

Please collect fresh blood for repeat test as the sample processed shows:

a. Sample was received in the laboratory after 24 hours of collection/clotted/hemolyzed (has broken RBC's in sample)
b. Abnormally low or high values
c. Others

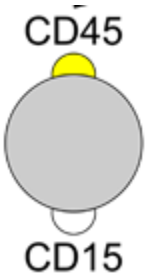
Date of reporting: _____

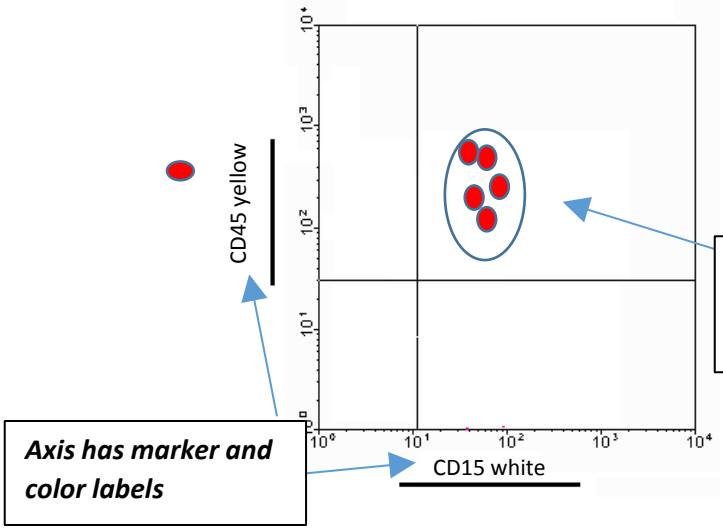
Analysis Question (4pts): Knowing that many resources say that AIDS develops once your CD4 count drops below 200 cells/mm^3 or when your CD4:CD8 ratio drops below 1, how can the reference ranges of the CD4:CD8 ratio for healthy men drop below 1?

Name(s): _____

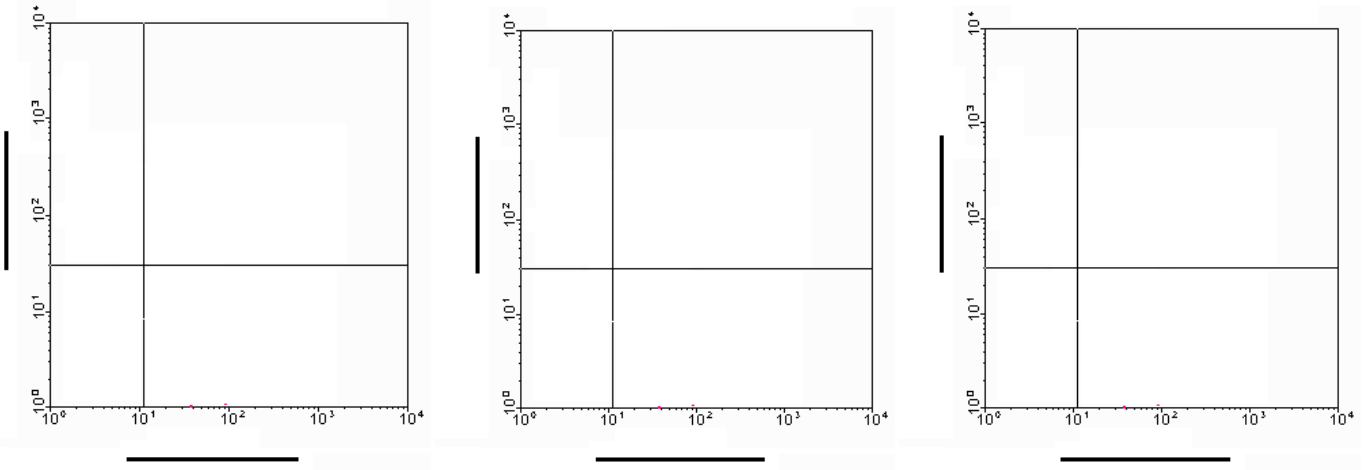
Flow Cytometry Raw Data Form

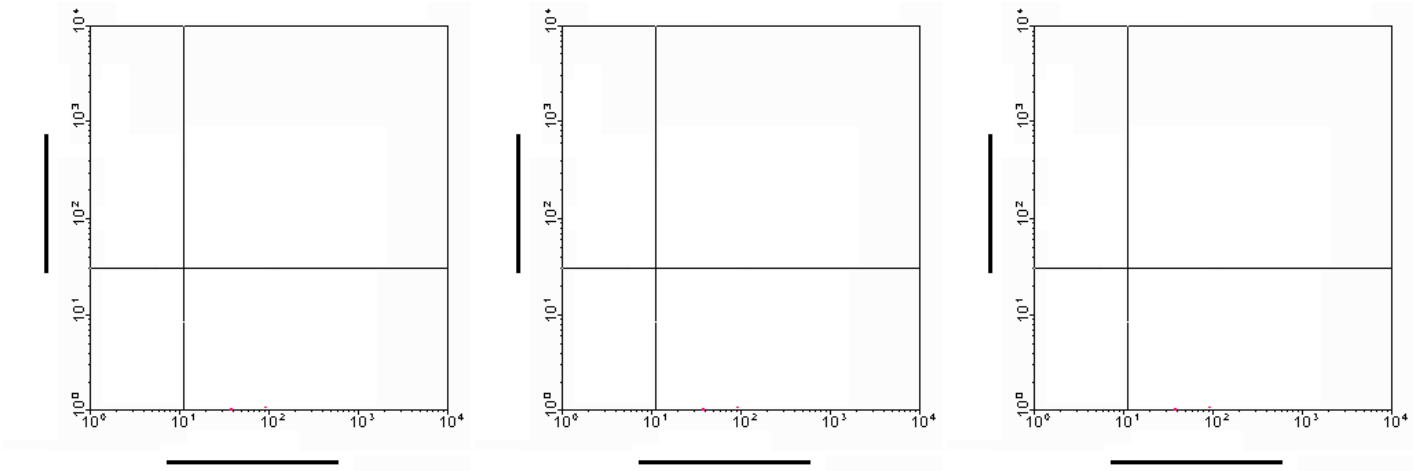
Directions: Generate Flow Cytometry graphs of the 2 different cells types given. Label the axis on the graphs with appropriate markers and color labels to give ALL possible combinations of the 4 markers and colors: CD45 yellow, CD19 green, CD4 blue, CD3 red. Draw the population in each quadrant where it would show up. Use the Immunophenotyping Scheme Key that came with your patient serum sample to guide your markers and colors on each cell type. An example is given below. NOTE: Some combinations may only give a double negative result. (2pts/graph=24 pts)



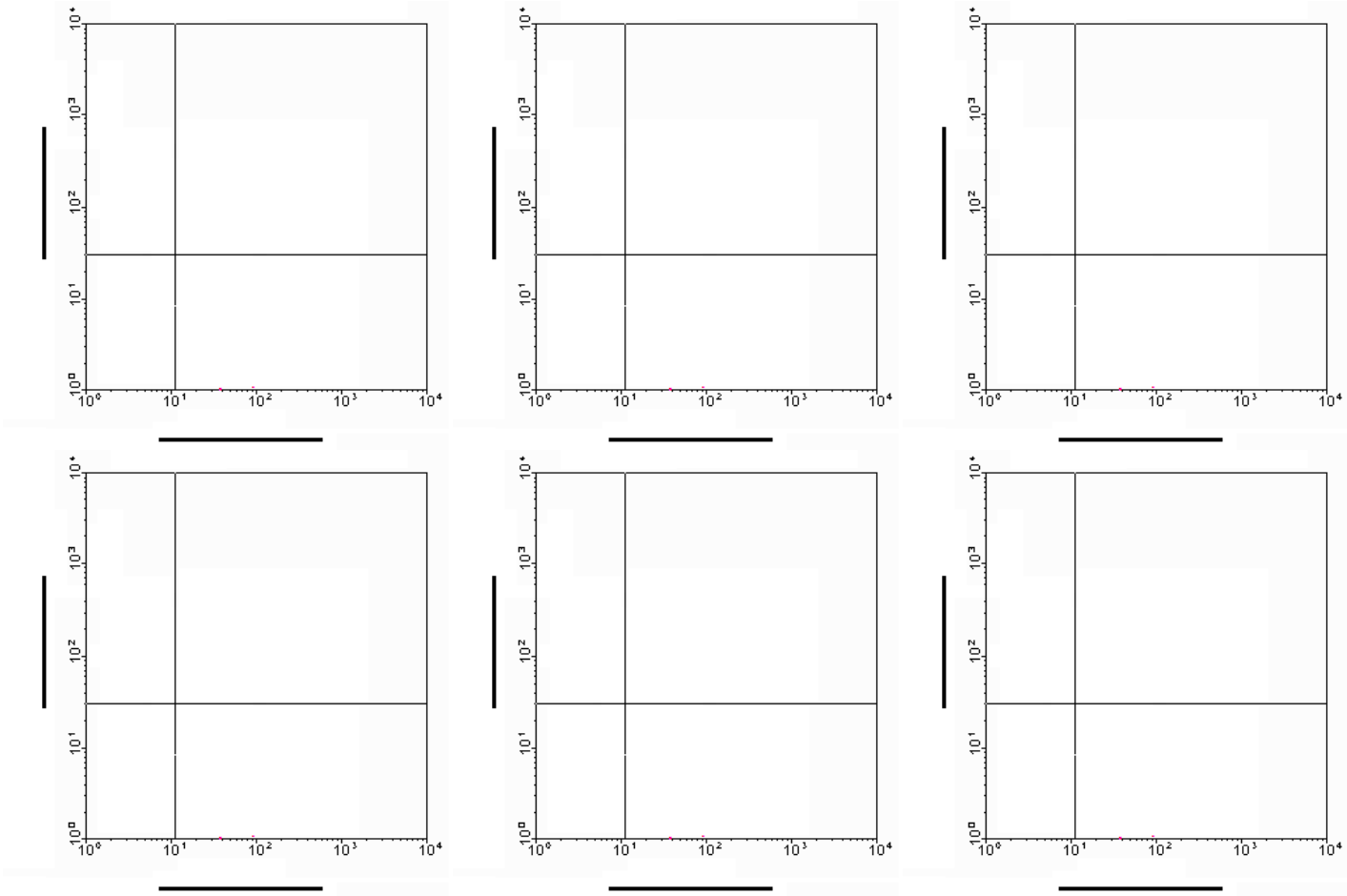


Cell Type 1: B-Lymphocytes





Cell Type 2: <u>Helper T-Lymphocytes</u> .



Immunophenotyping Scheme Key

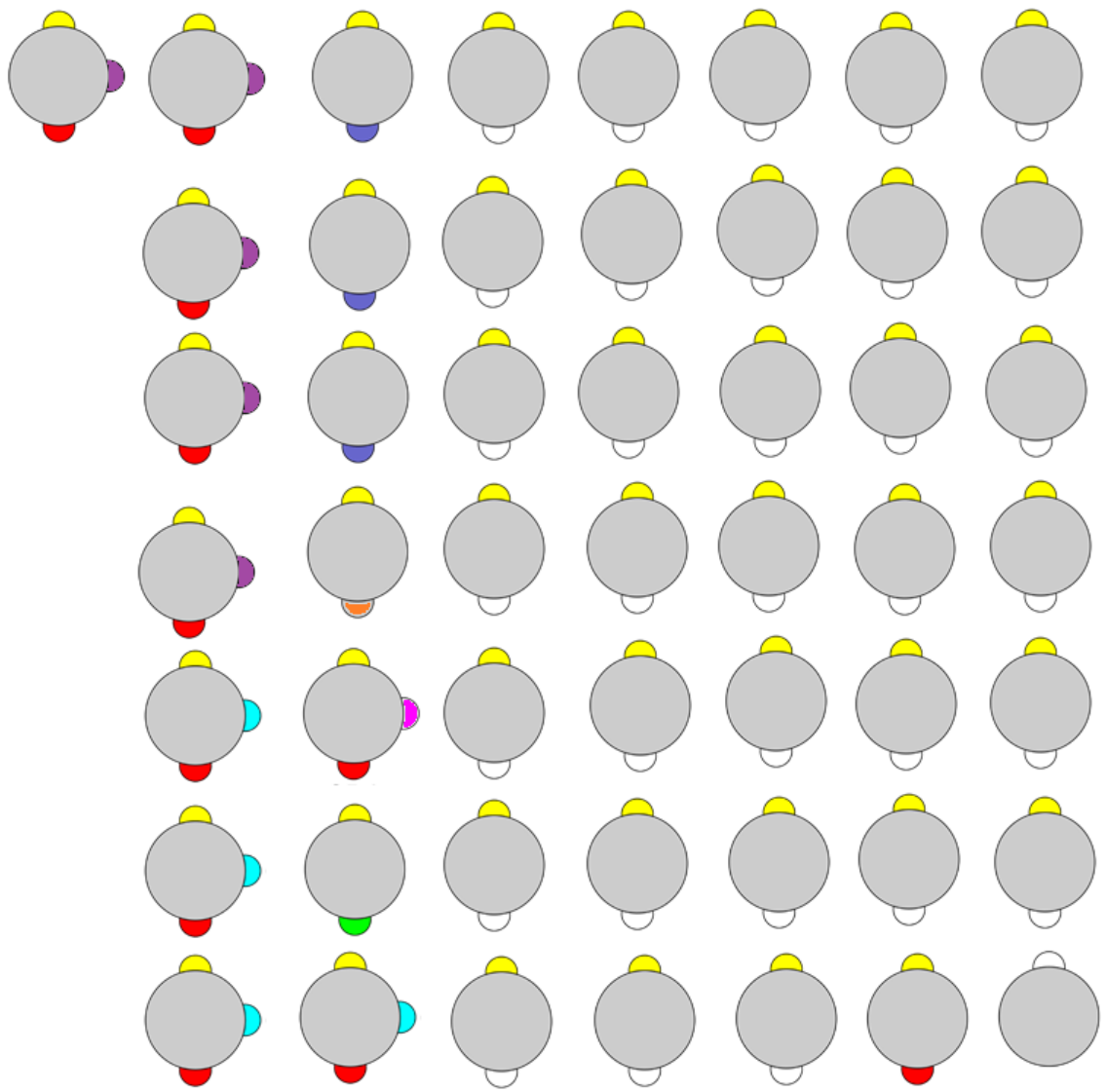
Fold and place in Ziploc bag- Do NOT cut.

https://upload.wikimedia.org/wikipedia/commons/thumb/a/a9/Cluster_of_differentiation.svg/1200px-Cluster_of_differentiation.svg.png

Patient Information:

Patient ID: 54321
Sex: F
Age: 16

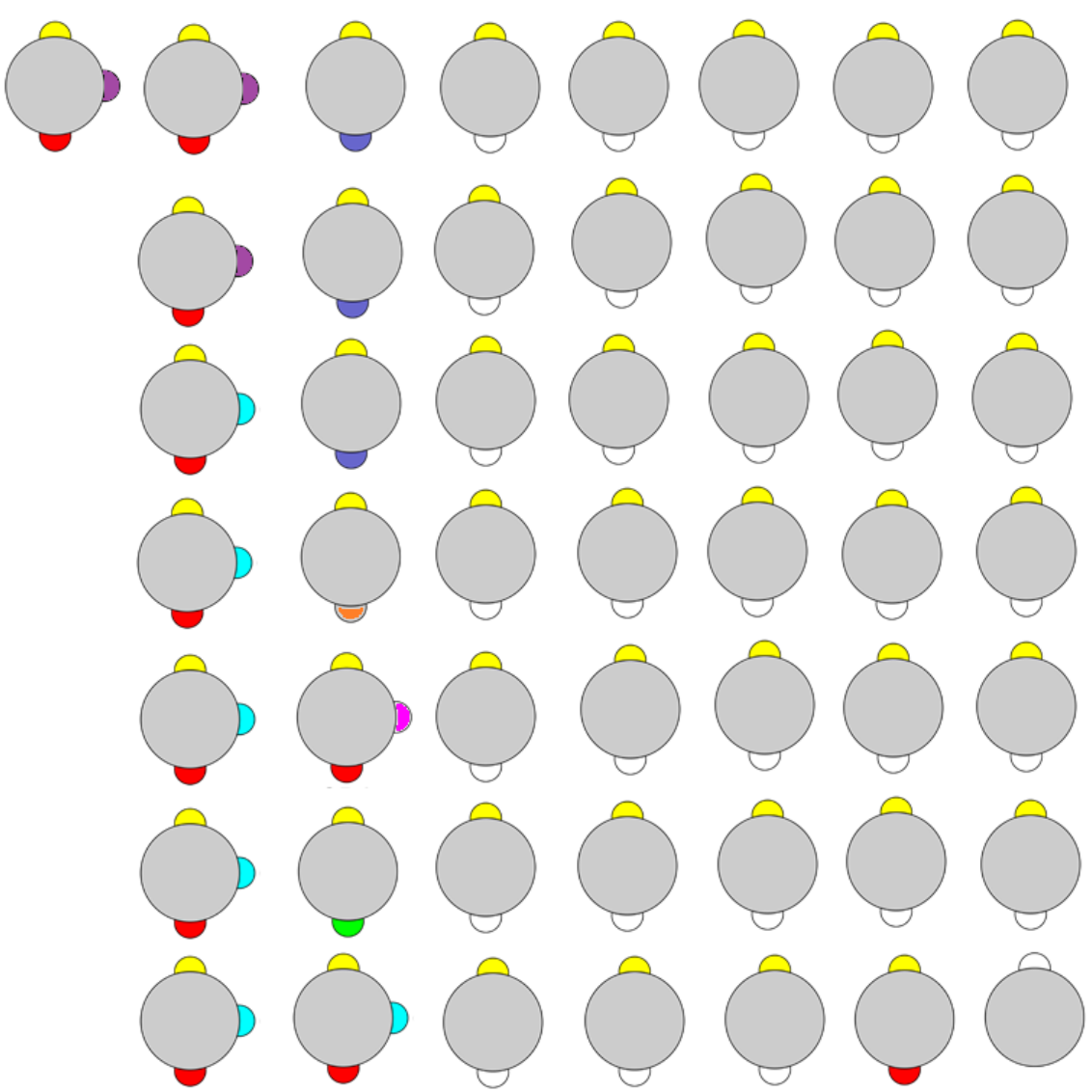
Requesting Doctor: Dr. Bottar
Clinic: Nationwide Children's Hospital



Patient Information:

Sex: M
Age: 56
Patient ID: 12345

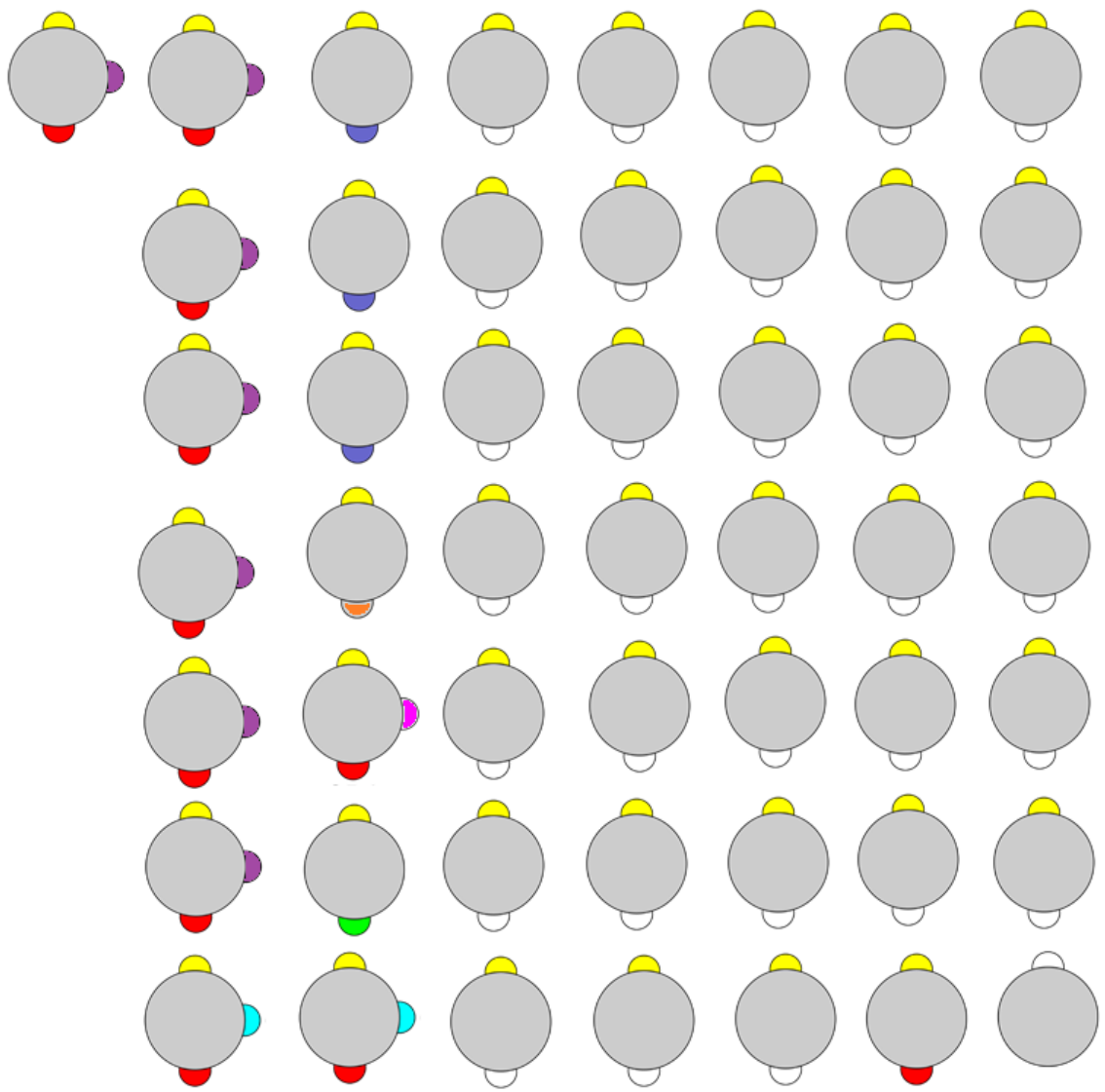
Requesting Doctor: Dr. Young
Clinic: OSU Wexner Medical Center



Patient Information:

Patient ID: 56789
Sex: F
Age: 22

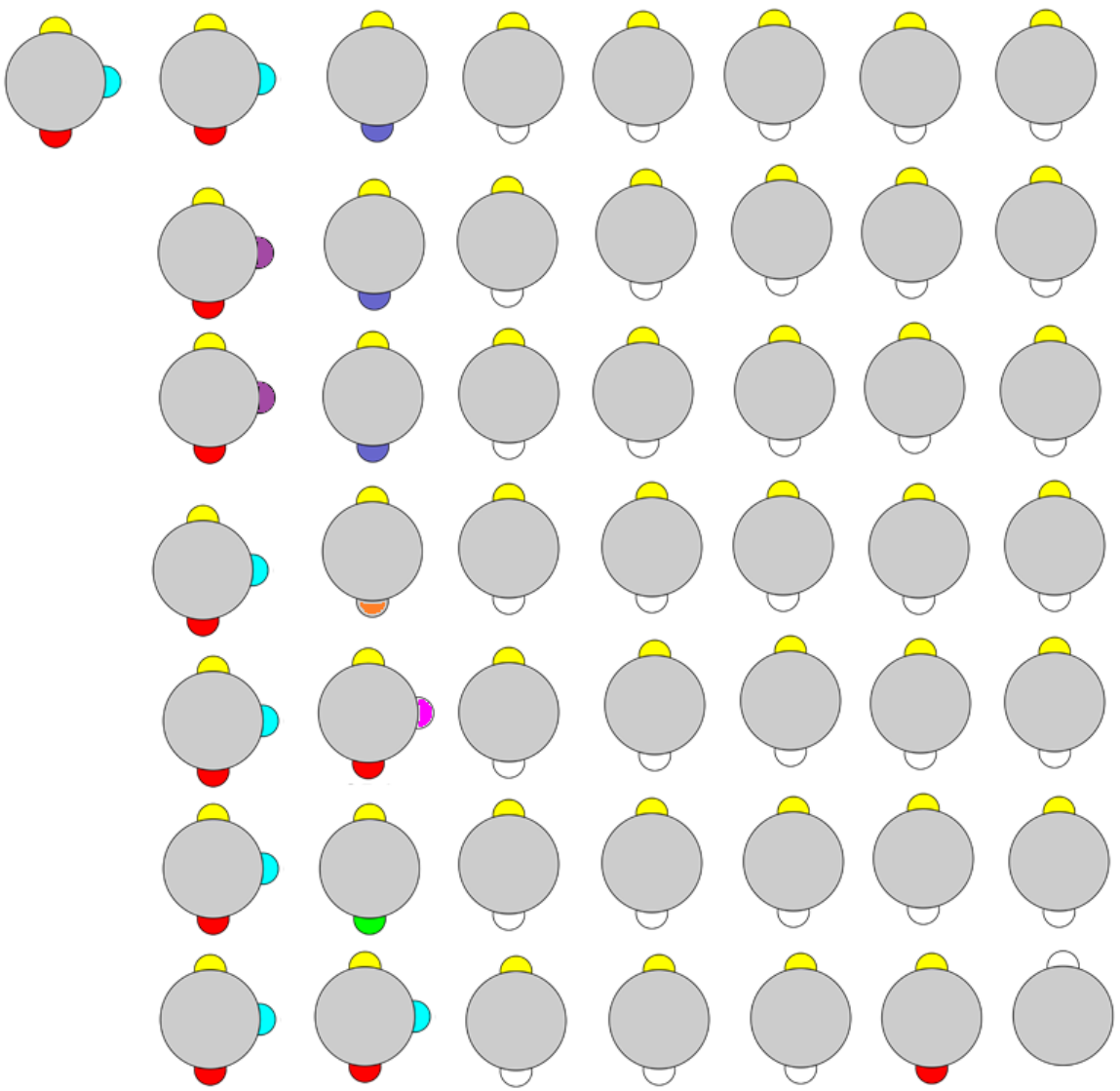
Requesting Doctor: Dr. White
Clinic: University Hospital



Patient Information:

Patient ID: 98765
Sex: M
Age: 30

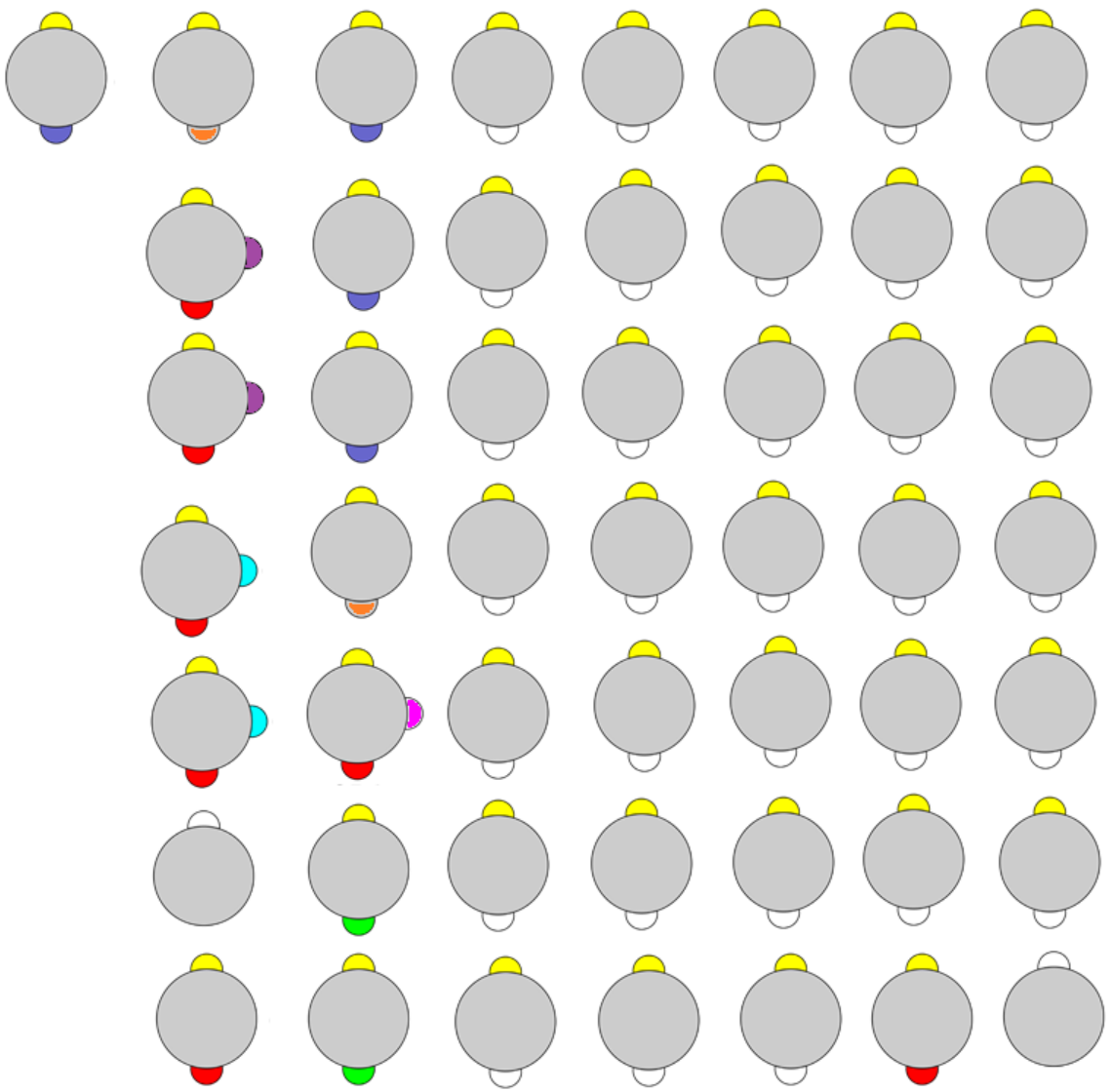
Requesting Doctor: Dr. Boyaka
Clinic: Cleveland Clinic



Patient Information:

Patient ID: 12389
Sex: M
Age: 15

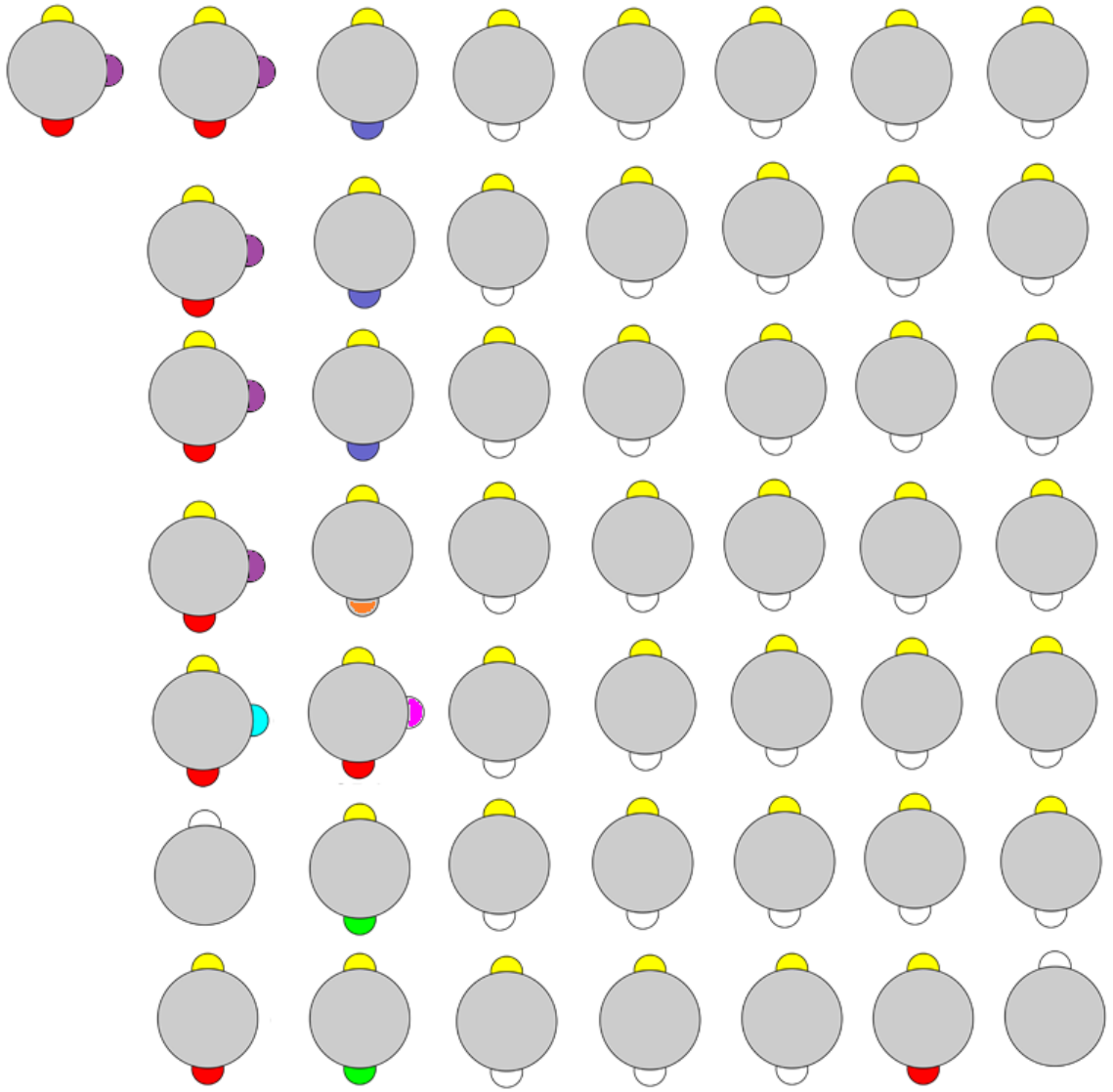
Requesting Doctor: Dr. Papenfuss
Clinic: Riverside Medical Center



Patient Information:

Patient ID: 98721
Sex: F
Age: 35

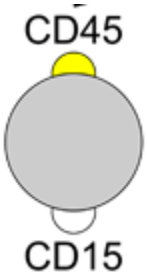
Requesting Doctor: Dr. Hill
Clinic: Med Express

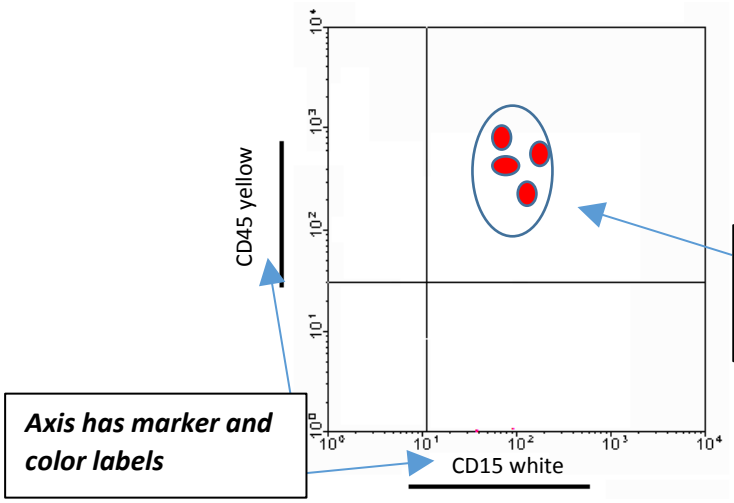


Name(s): _____

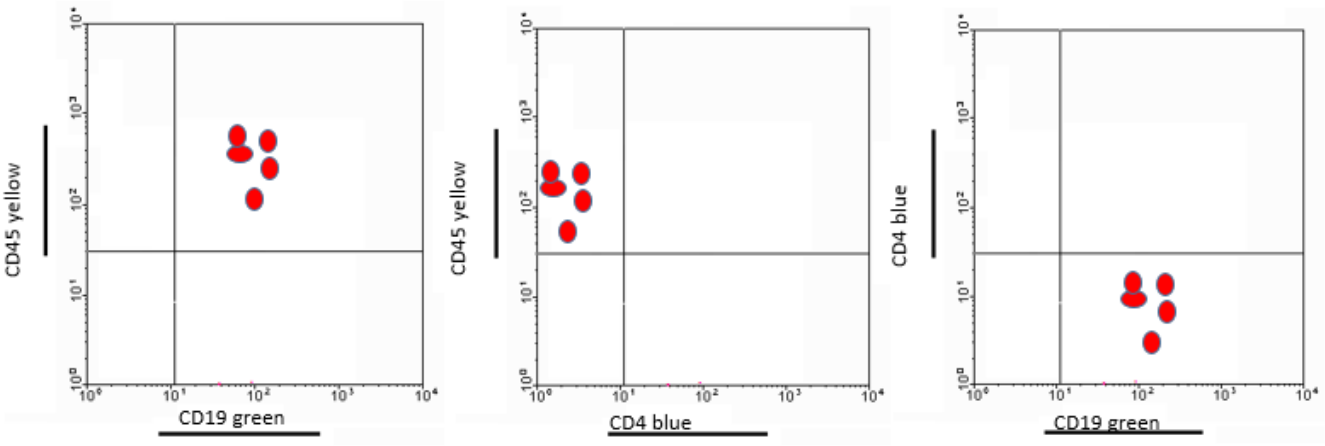
Flow Cytometry Raw Data Form-ANSWER KEY

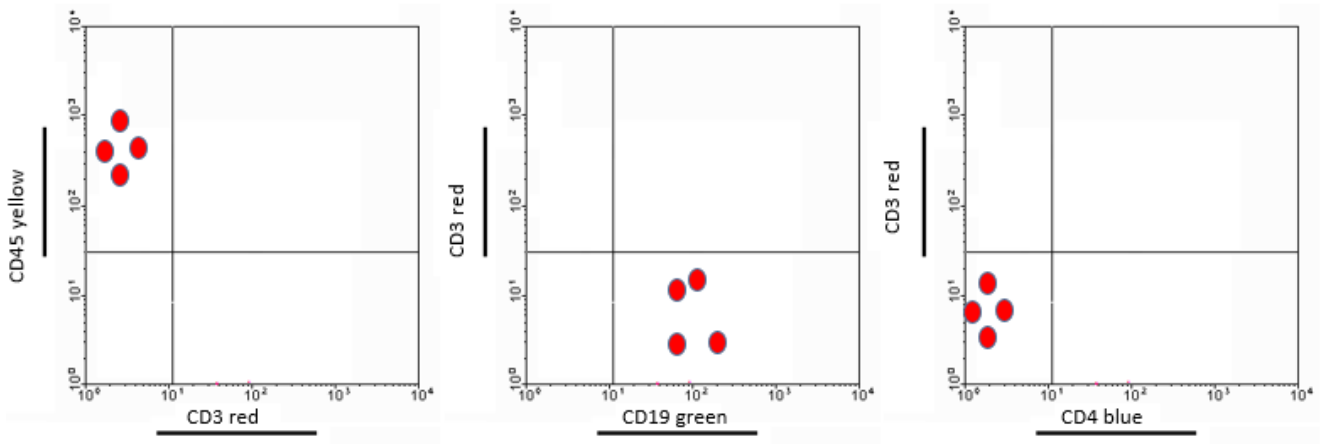
Directions: Generate Flow Cytometry graphs of the 2 different cells types given. Label the axis on the graphs with appropriate markers and color labels to give ALL possible combinations of the 4 markers and colors: CD45 yellow, CD19 green, CD4 blue, CD3 red. Draw the population in each quadrant where it would show up. Use the Immunophenotypeing Scheme Key that came with your patient serum sample to guide your markers and colors on each cell type. An example is given below. NOTE: Some combinations may only give a double negative result. (2pts/graph=24 pts)



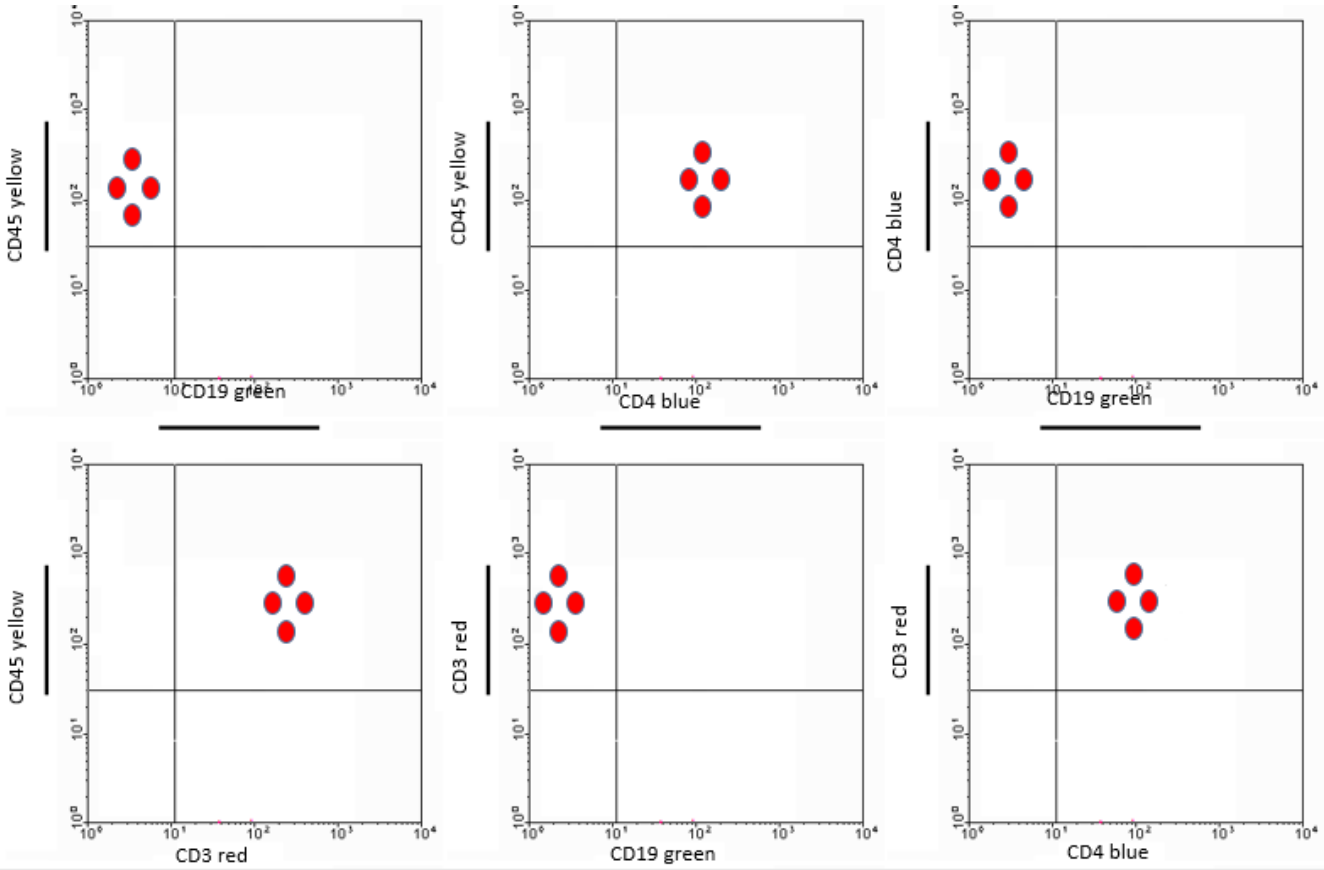


Cell Type 1: B-Lymphocytes graphs may vary based on axis labels .





Cell Type 2: <u>Helper T-Lymphocytes</u>.



xviii. Day 18: Flow Cytometry Raw Data Report

- Use the Immunophenotyping Scheme Key that was associated with the Day 2 activity to complete the Flow Cytometry Raw Data Report for 2 different cell types given.
- Collect both worksheets for analysis.

xix. Day 19: Antibiotic Resistance Review

- Antibiotic Resistance Assay Power Point and Discussion question
- Protocol Preview
- Pass out Rubric

Antibiotic Resistance Assay Lab Notebook Rubric

Topic	2	4	6
Title and page number	Title OR page numbers present	Both title and page numbers present	NA
Purpose	Purpose is included but incorrect	Purpose is included and correct	NA
Pre-lab questions (x2)	Prelab questions 1/3 complete or are very inaccurate	Pre-lab questions are 2/3 complete or have some inaccuracies	Pre-lab questions fully completed and accurate
Procedure (x2)	Procedure is incomplete and does not have clear numbered steps	Procedure is incomplete, but has clearly numbered steps	Procedure is clearly numbered and complete
Data collection and recording (x4)	There are no data tables AND incomplete/incorrect data is recorded	There are data tables, but they are not accurate or complete	There are complete and accurate data tables
Post lab questions (x2)	Post lab questions are 1/3 complete or very inaccurate	Post lab questions are 2/3 complete or have some inaccuracies	Post lab questions are complete and accurate
Conclusion	Conclusion is inaccurate or incomplete, missing 2 or more of the 4 parts: RE, PE, PA, and next steps	Conclusions is accurate but incomplete and is missing 1 of the 4 parts: RE, PE, PA, and next steps	Conclusion is accurate and complete with all 4 parts present.

xx. Day 20: Prepare petri dishes and E. coli broth culture
- Liquefy the solidified LB agar by microwaving on 50% power for 5 min with the cap of the bottle slightly loose.
- Let cool until it is slightly warm to the touch.
- Pour about 20 ml's of LB agar into 100mm petri dishes (enough to cover the bottom of the dish completely).
- Let solidify overnight at room temperature.
- Prepare E. coli broth culture by taking a single colony of E. coli from the stock petri dish and adding it to the 50 ml sterile LB broth. Incubate 24 hrs. @ 37°C on rocker

xxi. Day 21: Run the Antibiotic Resistance Assay.
- Inoculate LB Agar plates with 1 ml of E. coli from broth culture.
- Let sit for 15 min.
- Place one filter disk per antibiotic and a filter disk soaked in sterile DI water on each petri dish, for a total of 6 disks per plate. Repeat on 2 other petri dishes, for a total of 3 dishes. Triplicates are ran to obtain more accurate results.
- Incubate for 24 hrs. at 37°C.

xxii. Day 22: Analyze Antibiotic resistance plates
- Measure and record zones of inhibition in lab notebook.
- Average the data between the plates.
- Write conclusion on results and answer post lab questions.

xxiii. Day 23: Ouchterlony assay Intro
- Power Point discussion question
- Pass out Student Guide Packet
- Pass out Rubric
- Students start experimental design process- review old labs to show students what needs to be included in their experiment write up and brainstorm ideas

Ouchterlony Assay Lab Protocol Rubric

Topic	2	4	6
Title of Lab	Title OR page numbers present	Both title and page numbers present	NA
Background (x2)	Background has limited content and/or has many incorrect/non-relevant statements	Background contains the majority of relevant content and is explained well.	Background contains all of the relevant content and is explained fully.
Purpose	Purpose is included but incorrect	Purpose is included and correct	NA
Materials and Equipment	There are 3 or more materials or pieces of equipment missing from the list	1-2 materials or pieces of equipment missing from the list OR reagents are missing quantity, volume, and/or concentration	All materials and Equipment are listed and appropriate quantities, volumes, and concentration
Advance Preparation	Advanced preparation is addressed, but does not accurately reflect the necessities of their protocol.	Advanced preparations were accurately assessed and included.	NA
Experimental Parameters	Controls, variables, and time requirements are listed, but incorrect or incomplete (missing more than 2 things)	The listed controls, variables, and time requirements are correct, but incomplete, (missing 1-2 things)	Controls, variables, and time requirements are complete and accurate.
Procedure (x4)	Procedure is incomplete, does not have clear numbered steps, or is incorrect.	Procedure meets 2 of the following criteria: complete, clearly numbered, and correct	Procedure is clearly numbered, complete, and correct.
Data collection and tables (x3)	The data collection and table section is missing two of the following: 1. type of data you plan on collecting, 2. a reference scale for the data, 3. A neatly drawn table to record results in.	The data collection and table section is missing one of the following: 1. type of data you plan on collecting, 2. a reference scale for the data, 3. A neatly drawn table to record results in.	The data collection and table section contains all of the 3 requirements listed in the previous column.
Conclusion	The conclusion section is present, but is not clear on how students should analyze their data AND present their conclusions.	The conclusion section is present, but it is not clear how students should analyze their data OR present their conclusions.	Conclusion section is present and gives students a clear understanding of how students should analyze their data, draw and present conclusions.
Total			84

Ouchterlony Assay Lab Notebook/Results/Conclusion Rubric

Topic	2	4	6
Title and page number	Title OR page numbers present	Both title and page numbers present	NA
Purpose	Purpose is included but incorrect	Purpose is included and correct	NA
Procedure (x3)	Procedure is incomplete and does not have clear numbered steps.	Procedure is incomplete, but has clearly numbered steps.	Procedure is clearly numbered and complete.
Data collection and recording (x2)	There are no data tables and incomplete/incorrect data is recorded.	There are data tables, but they are not accurate or complete. Or there is no scale to determine relative size of line.	There are complete and accurate data tables with a scale present to determine the relative size of agglutination line.
Post lab questions (x2)	Post lab questions are 1/3 complete or very inaccurate.	Post lab questions are 2/3 complete or have some inaccuracies.	Post lab questions are complete and accurate.
Conclusion	Conclusion is inaccurate or incomplete, missing 2 or more of the 4 parts: RE, PE, PA, and next steps.	Conclusions is accurate but incomplete and is missing 1 of the 4 parts: RE, PE, PA, and next steps.	Conclusion is accurate and complete with all 4 parts present.
Total			56

xxiv. Day 24: Students complete experimental design rough draft and turn in for teacher review.

- Students need to have the number of plates needed for their experiment, amount of agar needed, their desired reagents they want to add to each plate, and the volume/concentration of each reagent.

xxv. Day 25: Students complete experimental design final draft and turn in for teacher review (can be typed if access to computers is available)

xxvi. Day 26: Students set up their Ouchterlony assay.

xxvii. Day 27: Students will read/record the results from their Ouchterlony Assay and write their conclusion.

xxviii. Day 28: Review for Summative Assessment.

xxix. Day 29: Students will take Summative Assessment.

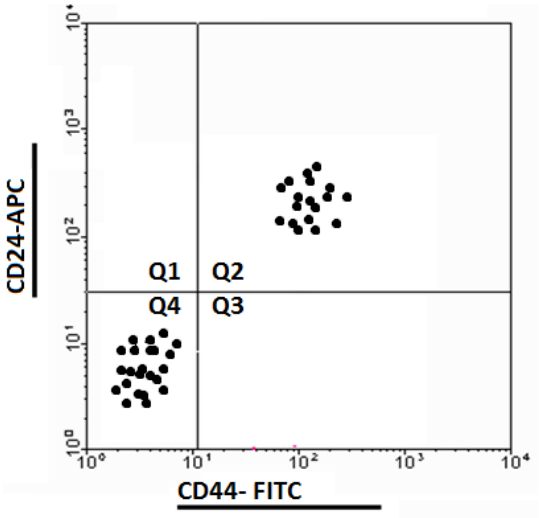
Immune System and Assays Summative Assessment

Name _____

Directions: *You are now experts on some very common immunology lab techniques. So, I just recommended you for a position in an immunology diagnostic lab. You have to read the scenarios below and answer the questions about each patient completely to the best of your ability. If you cannot diagnose the patients properly with the given information, you will be FIRED! I recommended you to this position, so you better do a good job! Good Luck!*

Patient Scenario 1:

1. *Patient 1 came into the doctor's office complaining of a lump that she found in her left breast during a routine self-examination. She said during her last self-exam 1 month prior it was not there. The doctor ordered a mammogram and a biopsy. He is afraid that it is breast cancer. You are told to run a flow cytometry experiment on the biopsied tissue. You did a literature search and found out that many breast cancers tend to have a phenotype of CD44+/CD24-. They become cancerous when they lose their CD24 expression. You ran the experiment and obtained the following results.*



Tell me the type of populations you have present in each quadrant. Based on the literature and your result, is patient 1 positive for breast cancer? Justify your answer. (4pts)

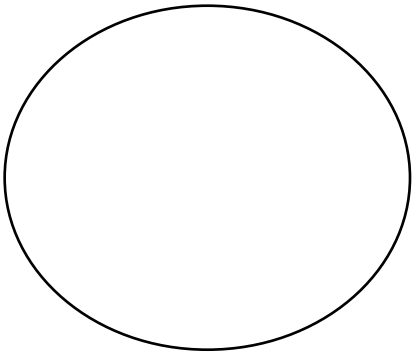
_____
_____
_____
_____

Patient Scenario 2:

2. *Patient 2 came into the doctor's office with a complaint of allergies, but he couldn't trigger what he was reacting to. He has symptoms on and off when visiting various friends and family, but he knows that he has a terrible reaction when he is inside his friend Tom's house. His friend has a cat, dog, ferret, and bird. His friend also smokes and is not very tidy, making his house smoky and dusty.*
   You ran the proper test and results came back positive for an allergy to dust and cats.

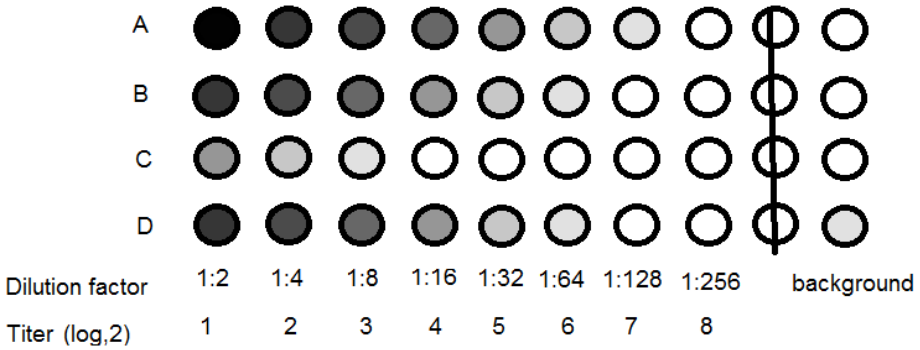
   Name the assay that you used to get these results (base it on our experiments we have done in class)? Draw out the plate, with results you obtained, and proper labels below. (6pts)

   Name of assay: _____



Patient Scenario 3:

3. *Patient 3 came into the doctor's office because he is getting a job at a construction company, and they require an up-to-date tetanus shot. He believes that he has had a tetanus booster within the past 10 years, but he cannot remember exactly when it was. To be safe, the doctor wanted to check his antibody titer. A protective effect of tetanus needs to an antibody titer of at least 4. Review the following lab results. Your patient's results are in row C.*

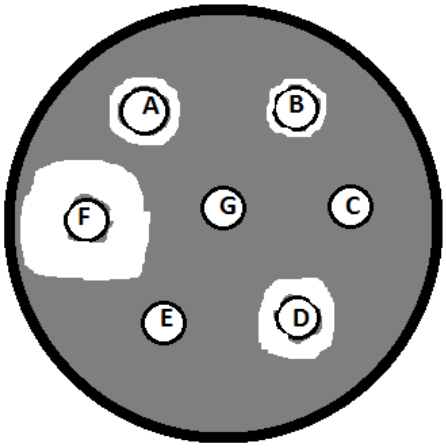


What type of assay did you run? Does he have to get a booster shot? Justify your answer. (4 pts)

_____
_____
_____
_____
_____
_____

Patient Scenario 4:

4. *A patient that has stage 4 cancer is undergoing strong rounds of chemotherapy on the 4th floor of the hospital where you work. Due to her harsh treatment, she has become immunocompromised. A MRSA outbreak has just occurred on the 3rd floor. They assigned you to the task of trying to find an antibiotic that will take care of the MRSA infection before it spreads to patient 4 and other immunocompromised people in the hospital. You ran an antibacterial resistance study with a variety of antibiotics on the Methicillin-resistant Staphylococcus aureus strain isolated in the hospital to find an antibiotic that can help take care of the outbreak. The results of your study are below.*



According to the results above, what antibiotic(s) could you use to treat the outbreak? Is there one that would be the best to use? Justify your answers. (4 pts)

_____
_____
_____
_____

5. Explain the difference between innate and adaptive immunity. Give examples of how each system fights pathogens and with what cells and/or mechanisms. (8 pts)

_____
_____
_____
_____
_____
_____

Matching: Match the following terms to the correct description of each immune cell. (2 pts each)

_____1. CD4 T-lymphocyte
_____2. B-lymphocyte
_____3. Plasma Cell
_____4. CD8 T-lymphocyte
_____5. Natural Killer Cell
_____6. Macrophage
_____7. Basophil
_____8. Neutrophil

A. A cell in your innate immunity that is the most abundant WBC and tends to be the first responder. It is phagocytic and releases toxins to destroy pathogen.
B. This cell is part of your adaptive immunity and is responsible for attacking cancer cells or cells infected with a pathogen.
C. This cell is part of your innate immunity and kills tumor cells and virus infected cells.
D. This cell is part of your innate immunity. It phagocytoses pathogens and presents them and recruits more immune cells.
E. This cell is part of your adaptive immunity. It secretes antibodies to its surrounds to attach to antigens.
F. This cell is part of your adaptive immunity and helps activate the B-cells to replicate.
G. This cell is part of your innate immune system. It is responsible for defense against parasites and releases histamines that cause allergic response.
H. This cell is part of your adaptive immunity and has antibodies that are locked to its surface that recognize and bind antigens.

Directions: Answer the following questions by picking the best answer and putting it on the line to the left of the question. (2 pts each)

_____1. Which of the following is NOT an anatomical barrier in the innate immune system?
   A. Sweat on your skin
   B. Mucus in your nasopharynx
   C. Kuepfer cells in your liver
   D. Tears form your eyes

_____2. Pick the most correct statement of the following options:

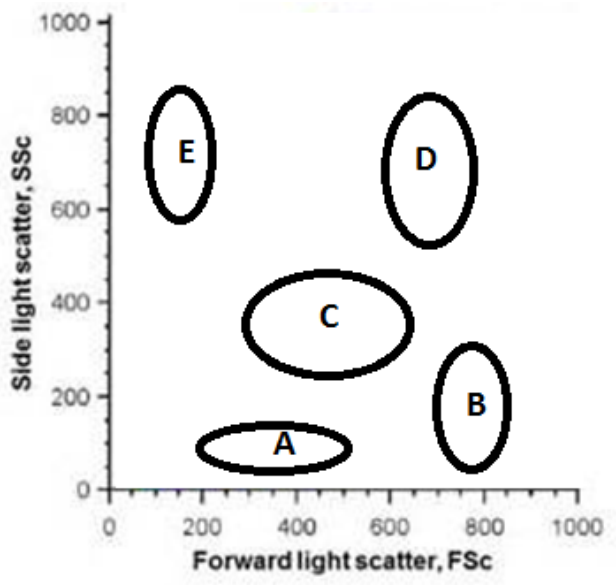
A. Innate and adaptive immunity occur at the same time when exposed to a new pathogen.
B. Innate immunity occurs first followed by adaptive immunity when exposed to a new pathogen.
C. Adaptive immunity occurs first followed by innate immunity when exposed to a new pathogen.
D. Neither occur when you are exposed to a new pathogen, you will die immediately since your body has no memory of this pathogen.

_____3. Why can't we make vaccines that always work against everything?

A. Because viruses and bacteria can mutate so that the antibodies which we originally produced don't always continue to recognizing them.
B. Our memory cells can dissipate over time if we are not occasionally re-exposed to the pathogen.
C. Because vaccines are expensive to produce and you need a large enough market to make your money back.
D. All of the above

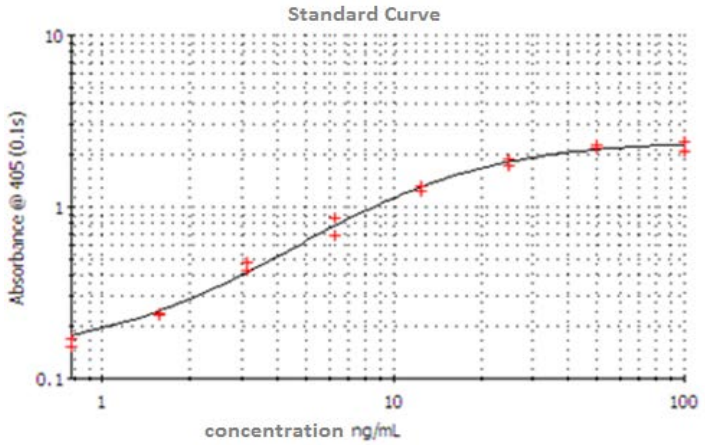
_____4. Where would very high granularity cells that are very small be on the following FSC (forward scatter) vs. SSC (side scatter) plot?

A. Area A
B. Area B
C. Area C
D. Area D
E. Area E



_____5. The standard curve shown below was generated on an ELISA plate. Using the standard curve, determine the concentration of antibody in a sample that has an average absorbance of 1.5 nm.

A. 12 ng/ml
B. 10.5 ng/ml
C. 100 ng/ml
D. 3 ng/ml
E. It does not fall on the curve



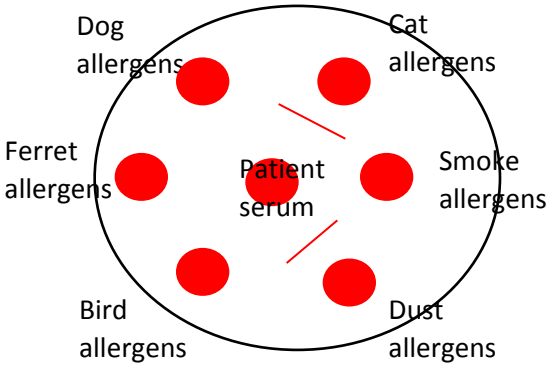
Immune System and Assays Summative Assessment- ANSWER KEY

Tell me the type of populations you have present in each quadrant. Based on the literature and you result, is patient 1 positive for breast cancer? Justify your answer. (4pts) I have a double negative population in quadrant 4 and a double positive population for CD44-FITC and CD24-APC in quadrant 2. There is no population on the graph that is CD44+ and CD24- at the same time, therefore, I believe that the patient does NOT have breast cancer.

Patient Scenario 2:

Draw out the plate, with results you obtained, and proper labels below. (6pts)

Name of assay: __ouchterlony_____



Patient Scenario 3:

What type of assay did you run? Does he have to get a booster shot? Justify your answer. (4 pts)

You ran an ELISA, and patient 3 does need a booster because his antibody titer is 2. The titer is one above the background and the 3rd well gives you a signal, but it is equal to the background signal. You need a titer of 4 to have a protective effect and you don't have that, so you need a booster shot to increase your antibody levels in your serum against tetanus.

Patient Scenario 4:

According to the results above, what antibiotic(s) could you use to treat the outbreak? Is there one that would be the best to use? Justify your answers. (4 pts)

You could use antibiotics A, B, D, and F. They all inhibited bacterial growth on the petri dish. Antibiotic F would work the best because it created the largest zone of inhibition of

the MRSA strain.

Explain the difference between innate and adaptive immunity. Give examples of how each system fights pathogens and with what cells and/or mechanisms. (8 pts)
Innate immunity is your body's first line of defense. It is non-specific, broad, and fast acting, but has no memory. It includes physical barriers such as skin, digestive enzymes, tears, and compliment proteins that puncture holes in cell membranes of pathogens. It also has some types of immune cells like macrophages (which phagocytose pathogens and present to adaptive immunity), neutrophils (phagocytose pathogens and release toxins to kill pathogens during a barrier breach), and NK cells (kill tumor and virus infected cells). Adaptive immunity is specific, slow initial response, antibody mediated, and has memory capabilities. It uses B-cells which create specific antibodies against the pathogen, plasma cells which release those antibodies to surrounding to bind to pathogens, T-cells which activate the B-cells and cytotoxic T-cells. Once pathogens are coated in antibodies, it makes it easier for macrophages to phagocytose.

Matching: Match the following terms to the correct description of each immune cell. (2 pts each)

_____9.	CD4 T-lymphocyte	F
_____10.	B-lymphocyte	H
_____11.	Plasma Cell	E
_____12.	CD8 T-lymphocyte	B
_____13.	Natural Killer Cell	C
_____14.	Macrophage	D
_____15.	Basophil	G
_____16.	Neutrophil	A

Directions: Answer the following questions by picking the best answer and putting it on the line to the left of the question. (2 pts each)

1. C
2. B
3. D
4. E
5. B

B. STUDENT GUIDE

I. Rationale:

i. Introduction to Immunology Part 1 and Cell Research Project

What is the immune system, what does it do, and how does it work? The immune system is your body's defense mechanism against any foreign invader such as bacteria and viruses that try to make you sick. It is like the military for your body. There are a variety of WBC's (White Blood Cells) in the human body that all serve different functions and in different places to help keep you healthy by fighting off pathogens. Each is a different branch of your body's military system. They all interact and communicate with each other to work together like branches of the military work together to destroy their target. You will become an expert in one type of immune cell by answering all of the questions outlined on your checklist. You will then teach the class about this cell and its interactions based on what you learned. You must include all of the check list information in your presentation. You can always include more. Make your presentation like you are trying to recruit us to your branch of the military!

ii. Introduction to Immunology Part 2 and Vaccines Article

It is very important to read current events in the news that is related to your health and wellness. But, you want to be able to dissect knowledge from a study on your own and not rely on the media to summarize it for you. Unfortunately, the media can misrepresent information. When Dr. **Andrew Wakefield** published his findings in 1998 that the MMR vaccine causes autism, the media ran with it even though no one was able to replicate and support his findings. He has since retracted his paper, because no one else can replicate his work and a conflict of interest was found in his study (lawyers that planned to sue big pharma companies approached him to prove that vaccines were detrimental). More information about this incidence can be found at: http://www.cnn.com/2011/HEALTH/01/05/autism.vaccines/index.html

Reading various research studies gives you the opportunity to see advances in science and medicine. Sometimes it can give you ideas or prior knowledge that will help you with a project you might be working on. You don't need to recreate the wheel or re-research something if it has already been done, proven, and published by ***multiple*** other scientists.

You are about to read current studies done in the field of vaccinations. These may or may not have been replicated yet, but they are all ways that scientists are trying to improve vaccination methods. Some of the studies have used different adjuvants to increase the immune response, different concentrations of antigen, different timing methods, or created a vaccine against something new. These papers summarize the purpose, procedure, and results of the studies. You will read the assigned article, dissect the important information, and present it to the class. You always want to read current studies to stay knowledgeable about advances in medicine.

The topic of vaccines is especially relevant to you because most of you have received vaccines in your lifetime. If they can find methods to improve vaccinations in ways where there would be fewer side effects, longer lasting immunity, or new vaccines against quickly mutating viruses, (such as HIV) it would greatly advance the field of medicine. Maybe there will be no fear of catching the flu or HIV in the future, like there is no fear of catching polio or measles now, if advances in vaccines continue.

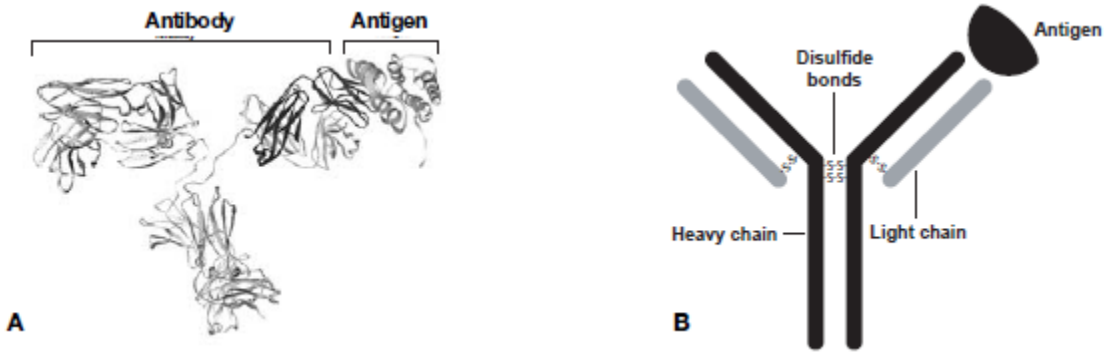
c. Quantitative ELISA Lab

Introduction

You are about to perform an ELISA or enzyme-linked immunosorbent assay; a test that detects antibodies in your blood to determine if you have been exposed to a disease. In this case we are testing multiple patients who have been vaccinated against MMR by various methods to determine which vaccination method produces the most robust immune response. The more antibodies against MMR, the better long term protection you will have. Patient summaries will be provided later.

When you are exposed to a disease agent, your body mounts an immune response. Molecules that cause your body to mount an immune response are called antigens, and may include components of infectious agents like bacteria, viruses, and fungi. Within days, millions of antibodies — proteins that recognize the antigen and bind very tightly to it — are circulating in your bloodstream. Like magic bullets, antibodies seek out and attach themselves to their target antigens, flagging the invaders for destruction by other cells of the immune system.

Over 100 years ago, biologists found that animals' immune systems respond to invasion by "foreign entities", or antigens. Today, antibodies have become vital scientific tools, used in biotechnology research, to optimize vaccination treatments, and to diagnose and treat disease. The number of different antibodies circulating in the blood has been estimated to be between 106 and 1011, so there is usually an antibody ready to deal with any antigen. In fact, antibodies make up to 15% of your total blood serum protein. Antibodies are very specific; each antibody recognizes only a single antigen.



A) Structure of IgG bound to the HIV capsid protein p24 as determined by X-ray crystallography (Harris et al. 1998, Momany et al. 1996). These structures can be downloaded from the Protein Data Bank (www.pdb.ufmg.br, (Berman et al. 2000) using the PDB identification codes 1IGY and 1AFV and manipulated using free online software such as Rasmol and Protein Explorer. **B)** A commonly used representation of an antibody bound to an antigen.

How Are Antibodies Made?

Scientists have learned to use the immune response of animals to make antibodies that can be used as tools to detect and diagnose diseases. The study of the immune system is called "immunology". Animals such as chickens, goats, rabbits, and sheep can be injected with an antigen and, after a period of time, their serum will contain antibodies that specifically recognize that antigen. If the antigen was a disease agent, the antibodies can be used to develop diagnostic tests for the disease. In an immunoassay, the antibodies used to recognize antigens like disease agents are called primary antibodies; primary antibodies confer specificity to the assay. When we are injected with a virus or other pathogen in a vaccine, our bodies mount an immune response and make antibodies specific to the virus or antigen that was administered to us.

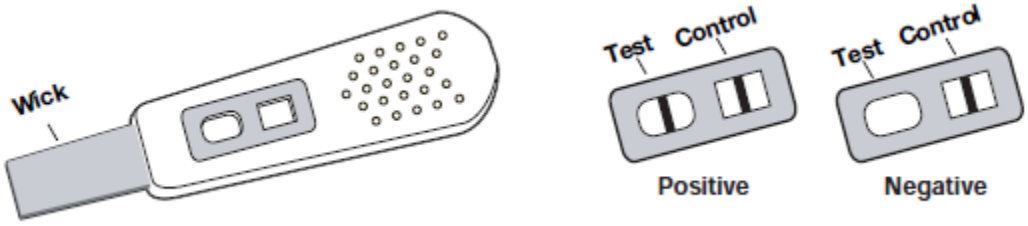
Other kinds of antibody tools, called secondary antibodies, are made in the same way. In an immunoassay, secondary antibodies recognize and bind to the primary antibodies, which are antibodies from another

species. Secondary antibodies are prepared by injecting antibodies made in one species into another species. It turns out that antibodies from different species are different enough from each other that they will be recognized as foreign proteins and provoke an immune response.

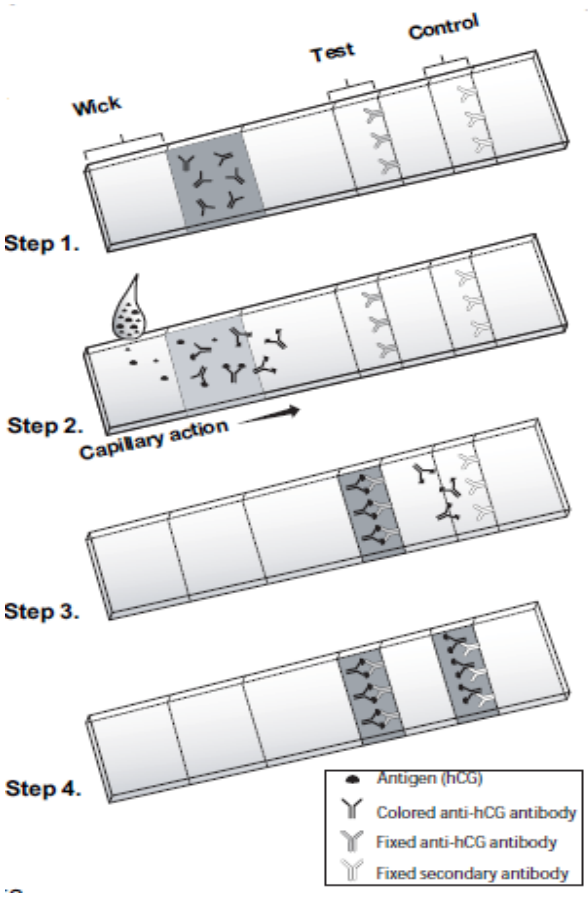
For example, to make a secondary antibody that will recognize a human primary antibody, human antibodies can be injected into an animal like a rabbit. After the rabbit mounts an immune response, the rabbit serum will contain antibodies that recognize and bind to human antibodies. The 2° antibodies used in this experiment are conjugated to the enzyme HRP which produces a blue color in the presence of its substrate, TMB. These antibody and enzyme tools are the basis for the ELISA.

Where Is ELISA Used in the Real World?

With its rapid test results, the ELISA has had a major impact on many aspects of medicine and agriculture. ELISA is used for such diverse purposes as pregnancy tests, disease detection in people, animals, and plants, detecting illegal drug use, testing indoor air quality, determining if food is labeled accurately, and to test antibody titers before administering a booster vaccination. For new and emerging diseases like severe acute respiratory syndrome (SARS), one of the highest priorities of the US Centers for Disease Control (CDC) and the World Health Organization (WHO) has been to develop an ELISA that can quickly and easily verify whether patients have been exposed to the virus. Over-the-counter kits that are based on the same principles as the ELISA include home pregnancy and ovulation tests, and tests for the presence of illegal drugs like marijuana and cocaine.



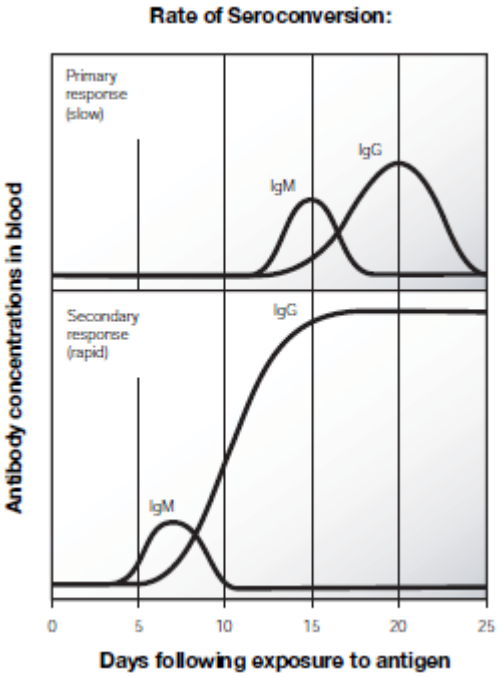
Some tests give positive or negative results in a matter of minutes. For example, home pregnancy dipstick tests detect levels of human chorionic gonadotropin (hCG), a hormone that appears in the blood and urine of pregnant women within days of fertilization. The wick area of the dipstick is coated with anti-hCG antibody labeled with a pink compound (step 1). When the strip is dipped in urine, if hCG is present it will bind to the pink antibody, and the pink hCG-antibody complex will migrate up the strip via capillary action (step 2). When the pink complex reaches the first test zone, a narrow strip containing an unlabeled, fixed anti-hCG antibody, the complex will bind and concentrate there, making a pink stripe (step 3). The dipsticks have a built-in control zone containing an unlabeled secondary antibody that binds unbound pink complex (present in both positive and negative results) in the second stripe (step 4). Thus, every valid test will give a second pink stripe, but only a positive pregnancy test will give two pink stripes.



The Immune Response

Following exposure to a disease, the immune system produces antibodies directed against the disease antigens. The typical immune response follows a predictable course. First, the body

produces a type of antibody called immunoglobulin M, or IgM. Within a week of disease exposure, IgM can be detected in the blood. Approximately 3 weeks after that, IgM levels drop and levels of another immunoglobulin, IgG, begin to rise. If there is no further disease exposure, the levels of IgG drop rapidly after a few days. The second time the body is exposed to a disease, the immune response is stronger and faster than the first time. This is the principle behind vaccination (causing the primary response) and booster shots (causing the secondary response). By exposing you to a disease agent that can't harm you (for example, an injection of inactivated influenza virus), your doctor is ensuring that when you are exposed to active flu virus, your immune system will respond rapidly to the invader.



This ELISA protocol is designed to detect the presence of antibodies circulating in the blood that have been produced in response to exposure to a specific disease. The technique is based on the fact that antibodies produced by your body in response to a disease antigen will bind tightly to that antigen — even in a test tube. All that is needed is a source of pure antigen to capture the antibody of interest and a way to visualize the result.

Controls in Immunoassays

For any immunoassay to be valid, it must include both positive and negative controls, i.e., samples that will give known results. Controls are always run side by side with actual samples. If you do not run a positive control and the experiment gives negative results, how can you be sure the results are truly negative? What if the assay simply did not work? If a positive sample gives a negative assay result, it is called a **false negative**. Conversely, if you do not run a negative control and the experiment gives all positive results, how can you be sure the results are truly positive? What if the assay was

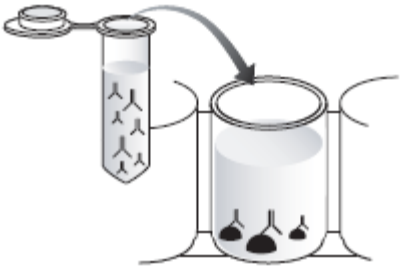
contaminated with antigen? If a negative sample gives a positive assay result, it is called a **false positive**.

Why Do We Need Controls?
Control samples are necessary to be sure your ELISA is working correctly. Many
diagnostic assays give a percentage of false positive or false negative results, so confirmation of diagnosis by a second type of assay is important. For example, immunoassays for antibodies to human immunodeficiency virus (HIV) may give either false positive or false negative results. False positives can result from recent vaccinations, and false negatives can result from immunosuppression (e.g., from drugs given after transplants) or from administering the test too soon after infection with HIV. (Antibodies against HIV do not appear until some weeks after HIV infection; the appearance of antibodies is called seroconversion.) Because of this, positive HIV ELISA tests are always confirmed by performing a western blot.

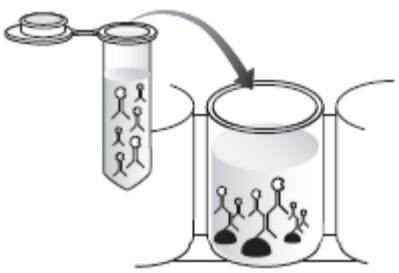
Controls are also needed to guard against experimental errors. There can be problems with reagents, which can degrade due to age or poor storage conditions. Technicians can make mistakes by choosing the wrong reagents, making errors in dilutions or in pipetting, or failing to remove unbound reagents. Poor record keeping is another source of false assay results. Most of these possibilities can be checked for within the assay with the appropriate controls.

Now let's put this all together.

The main steps in the ELISA antibody test are:



1. Add purified disease antigen to the wells of a microplate strip. Incubate for 5 minutes to allow proteins to bind to the plastic wells via hydrophobic interaction. This is called an immunosorbent assay because proteins adsorb (bind) to

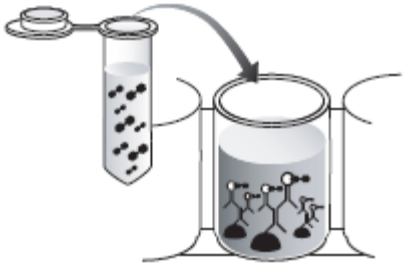


the plastic wells.

2. Add your serum sample and control samples to the wells and incubate. Serum contains millions of different types of antibodies, but only if your serum contains antibodies that were produced in response to the disease will antibodies bind to the antigen in the wells.

3. Detect the serum (primary) antibodies with HRP-labeled secondary antibody. If serum antibodies have bound to the antigen, the secondary antibodies will bind tightly to the serum antibodies.

4. Add enzyme substrate to the wells, wait 5 minutes, and evaluate the assay results. If the primary antibody was present in your serum sample, the wells will turn blue. This is a positive diagnosis. If the wells remain colorless, the primary antibody was not present in your serum sample, and the diagnosis is negative.



iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

You just got hired in a clinical diagnostics lab. Part of your job is to run patient samples that have been stained with known cell markers tagged with fluorochromes to help diagnose various diseases such as cancer, HIV/AIDS, autoimmune disorders, etc. You just completed training on the flow cytometer (notes and videos we did in class) and it is time to run your first patient sample. Today, an HIV positive patient got their blood taken to see if the viral infection has progressed to AIDS. HIV (aka Human Immunodeficiency Virus) attacks CD4 T-cells. The looped surface protein, glycoprotein 120 (gp120) on HIV attaches to the CD4 receptor and another CD4 T-cell co-receptor such as CCR5. The membranes then merge, releasing viral content into the host T-cell. The virus then inserts its viral RNA into the host genome by reverse transcribing it into viral DNA. The host cell then transcribes and translates the necessary viral proteins to synthesize new viruses. These proteins and molecules gather at the host's plasma membrane and bud off the cell. The viral envelope is now composed of the host cells plasma membrane. When enough viruses

bud off the host cell, the plasma membrane becomes compromised and causes cell death. Between cell death by loss of membrane integrity, increase in viral load, and cell death by other mechanisms in the immune system that recognize virally infected cells or internal controls regulating apoptosis, the CD4 T-cell number drops over time. This drop in CD4 T-cells has a detrimental effect on the immune system and its ability to protect your body from infections and cancer.
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CD4 T-cells are also called Helper T-cells (Th cells). They activate B-cells to produce antibodies against specific pathogens in the humoral branch of adaptive immunity. They also activate CD8 T-cells to differentiate into cytotoxic T-lymphocytes (CTLs) in the cell mediated branch of the adaptive immune response. Because CD4 T-cells have to recognize a variety of specific antigens with their T-cell receptors (TCR's) that are presented by APCs (antigen presenting cells, such as macrophages and dendritic cells), you need a wide variety of CD4 T-cells available, each with unique recognition epitopes on their TCR's, to match the vast array of possible antigens. Without a large enough quantity of CD4 Th cells, the bodies adaptive immune response cannot respond effectively to constant bombardment by various pathogens.

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According to AIDS.gov, the average number of CD4 T-cells in healthy individual's ranges from 500-1500 cells/mm^3. The diagnosis of AIDS is given when the CD4 T-cell population drops below 200 cell/mm^3. It has been found that HIV infected patients start to develop symptoms related to AIDS such as nausea, fever, and other signs of illness when their CD4 T-cell number drops below 200 cells/mm^3. But, CD4 and CD8 T-cell numbers vary greatly between healthy individuals, even dropping below the reference ranges provided. These differences can be based on sex, age, other health issues, genetic predispositions, and a variety of other factors. Therefore, clinical lab technicians run assays that also look at total WBC count, CD4%, CD8%, and the CD4:CD8 ratio, not just CD4 and CD8 cell counts. Although there is still a range of values that healthy individual's fall into, these numbers tend to be more reliable and consistent throughout the population than absolute cell counts. According to AIDS.gov, when the CD4:CD8 ratio drops below 1, it qualifies an HIV infected individual to be diagnosed with AIDS.

You will use the provided forms with reference ranges, serum sample bag, and the immunophenotyping schematic key to diagnose your patient with AIDS or to justify their current health. It is important to understand how lab tests are run and reported. You will get a variety of lab tests done in your lifetime, so it is a useful skill to learn how to read and interpret the results from those tests.

v. Antibiotic Resistance Assay

Since the introduction of antibiotics into the healthcare market in the early 1940's there has been increased use due to their great effects. Since their discovery and implementation, overall health, wellness, and lifespan has increased. But, since that time antibiotics have been abused with overuse. Due to the overuse of these antibiotics, the microorganisms that they once were effective at targeting have evolved to resist the drugs. As of 2013, the CDC has identified 18 antibiotic resistant microorganisms of concern, and that number is on the rise. Outbreaks are occurring at major medical facilities like hospitals and nursing homes all around the US. When these outbreaks occur it is important to identify the organisms and treat them with specific antimicrobial agents to prevent further mutation of the resistant population.

In order to decide which antibiotic or antimicrobial agent would be best suited in different outbreak situations, antibiotic resistance assays are run. In an antibiotic resistance assay, the resistant microorganism is cultured with a variety of antimicrobial agents of interest. During its incubation, growth of the microorganism will be inhibited if the antimicrobial agent is effective against that particular microorganism. The larger the zone of inhibition around the antimicrobial agent soak filter disk, the more effective the drug is at preventing growth and proliferation. An antibiotic resistance assay is pictured below. Running this assay before random treatment is a much quicker, cheaper, and safer way to try and eradicate the outbreak. It is cheaper because you are not spending hundreds to thousands of dollars on ineffective antibiotics to treat people. It is safer because people are not getting unnecessarily treated with drugs that may have detrimental side effects to the patients such as diarrhea or allergies. It is quicker because you can see resistivity or sensitivity results within 24 hours opposed to a few days in patients. https://nanowatertreatment.wikispaces.com/file/view/Disk_Diffusion_As

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We have had a number of MRSA (methicillin-resistant staphylococcus aureus) cases at our school from sports injuries where the skin has been punctured, breaking one of our anatomical barriers that protects us from pathogen invasion. Even though these students were not immunocompromised at the time of initial infection, it still took a great deal of time to rid themselves of these bacterial infections due to their resistance to a wide spectrum of antibiotics. Researchers are currently fighting the clock to discover and develop new antibiotics to help treat these resistant organisms. The majority of antimicrobial agents are actually derived and isolated from other organisms that produce the product naturally such as plants and other microorganisms. Due to this, researchers are currently looking at a variety of plants and organisms located in the rain forest because it is one of the most diverse and least explored places on earth.

St. Elizabeth's hospital called our biotechnology lab to run an antibiotic resistance assay for them on a new MRSA strain that they isolated from one of our students' sports injuries. While he was in the hospital his MRSA infection spread to 4 other patients in his wing. The St. Elizabeth's staff is concerned that it will continue to spread if not controlled. Your job is to find any and all antibiotics that are effective at inhibiting the growth of the MRSA strain isolated at the hospital before it spreads to more immunocompromised patients on other floors, such as our HIV/AIDS patients from the last lab.

vi. Ouchterlony Assay Development

Many people have allergic responses to some antigen nowadays. We all know someone with an allergy to at least one of the things listed here: pollen, animal dander, smoke, dust, peanuts, bee stings, and the list could go on and on and on. Allergies to antibiotics can be life threatening because they are administered in a fashion that they are required to travel throughout your body to the site(s) of infection. This means that you may experience an allergic response throughout your entire body. This is unlike most dust or pollen allergies that usually just make you sneezy and your eyes itchy at the site of antigen contact (your mucus membranes, which also act as anatomical barriers). Bee stings and food allergies can also be severe because they break or surpass the anatomical

barriers and get into your susceptible tissues.

It is important to understand how allergies work. Allergies are caused by an immune response of IgE binding to antigens, such as pollen, antibiotics, etc. When IgE binds these antigens, it sets off a cascade of inflammatory responses which cause, redness, itching, swelling, runny nose, etc. Some of these allergic responses are non-life threatening, but some are. If your allergic response is severe enough, your airway can swell completely closed, causing you to suffocate. Epi pens are administered to people with severe allergies to act as a short term steroid response to prevent this from happening. These patients still need to go straight to the hospital if they encounter one of their severe allergens to be monitored.

When antibiotic allergies are known, it is important to ensure that the prescribed antibiotic does not induce an allergic response. You are trying to heal the patient, not cause more harm.

There are 6 patients at St. Elizabeth's hospital that have known antibiotic allergies. Based on your Antibiotic Resistance testing results to the isolated MRSA strain you will develop an experiment to ensure these patients do not have allergies to any of these antibiotics. You will run an Ouchterlony assay. But, this is the first Ouchterlony this hospital has had to do, so there is no protocol on file. Your manager does not have time to create the protocol herself, so you are put to the task of creating the protocol and submitting it to your supervisor for approval. Once approved, you will run the assay that you developed, make a data table that you can record the results in, and draw conclusions about your patients' sample. You will only be given 1 patient at a time, so write the protocol for 1 complete assay. You can use the power point notes on this assay and other resources to help you develop this protocol. You will submit a rough draft for approval before you make the final copy and complete the assay in order to produce the best results possible with minimum error.

II. Materials (per group):

i. Introduction to Immunology Part 1 and Cell Research Project

- Checklist with assigned WBC
- Scoring Rubric
- Computer with internet
- Projector
- White board and dry erase markers

ii. Introduction to Immunology Part 2 and Vaccines Article

- Poster board
- Articles
- Markers

iii. Quantitative ELISA Lab

Laboratory Guide

Student Workstation Checklist

One workstation serves 4 students.

Item (label)	Contents	Number	(✓)
Yellow tube (STAN)	standard (250 µl)	1 -1.5ml tube	☐
Yellow tube (P1)	Patient 1 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P2)	Patient 2 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P3)	Patient 3 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P4)	Patient 4 (250 µl)	1 -1.5ml tube	☐
Yellow tube (P5)	Patient 5 (250 µl)	1 -1.5ml tube	☐
Violet tube (+)	positive control (350 µl)	1 -1.5ml tube	☐
Blue Tube (-)	negative control (350 µl)	1 -1.5ml tube	☐
Clear Test Tube (AG)	Purified antigen (5 ml)	1 -**5ml** tube	☐
Clear Test Tube (SA)	Secondary Antibody (5 ml)	1 -**5ml** tube	☐
Clear Test Tube ***covered in foil*** (SUB)	Enzyme Substrate (5 ml)	1 -**5ml** tube	☐
1 -96 well ELISA plate		1	☐
50 µl fixed volume micropipette OR 20-200 µl adjustable micropipette		1	☐
Yellow Tips to fit micropipette		1 box	☐
Disposable plastic transfer pipet		1	☐
120-150 ml wash buffer in 200 ml beaker	1x PBS with 0.05% Tween 20	1	☐
Large stack of paper towels		2 stacks	☐
Black marking pen		1	☐

iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

- Ziploc bag with cut out assigned stained patient serum sample and Immunophenotyping Scheme Key
- HIV/AIDS Testing- Sample Transit, Receipt, and Report Form
- Calculator
- Flow Cytometry Raw Data Form

v. Antibiotic Resistance

- 3- 100mm petri dishes filled with LB agar
- Sterile transfer pipet.
- E. coli Broth culture
- Glass plate spreader OR wire inoculating loop
- 3 Blank filter paper disks soaked in sterile DI water
- 3 ampicillin filter paper disks 10 ug
- 3 chloramphenicol filter paper disks 30 ug
- 3 penicillin filter paper disks 10 ug
- 3 streptomycin filter paper disks 10 ug
- 3 tetracycline filter paper disks 30 ug
- Antibiotic disk dispenser
- Incubator set to 37°C
- Googles
- Gloves
- 10% bleach
- 70% ethanol
- Paper towels
- Permanent marker

vi. Ouchterlony Assay Development

- Patient Serum (they will choose Patient A- Patient F)- 700 µl (they will determine volume based on triplicates of experiment, adding 200 µl in each corresponding well, and adding extra volume in case of pipetting error).
- 3 known antibiotic solutions- 70 µl (they will determine volume based on triplicates of experiment, adding 20 µl in each corresponding well, and adding extra volume in case of pipetting error).
- 0.9% Agarose in PBS with 8% sodium chloride -80 mls (based on

triplicates of experiment with about 20-25 mls media per plate, with extra in case of error in pouring)

- Sterile transfer pipets- 6 (each antibiotic gets 1, the patient serum gets 1, the negative control gets 1, and 1 gets cut to suck out agar to make wells)
- 35mm x15mm petri dishes, sterile- 3 based on triplicates of experiment
- Phosphate Buffered Saline Solution- 70 µl for negative control for each of the 3 plates.
- Permanent marker- to label plates
- Gloves- safety

vii. Immune System and Assays Summative Assessment

1. Test

III. Procedure

i. Introduction to Immunology Part 1 and Cell Research Project

1. Include the criteria listed on the checklist in your presentation. Keep track by checking off the criteria as you go to ensure it is all present.

ii. Introduction to Immunology Part 2 and Vaccines Article

1. Read assigned article in your group.
2. Write the following information on your large sheet of presentation paper.

a. Title of article (2 pts)
b. Date published (2 pts)
c. Author or editor (2 pts)
d. Journal (2 pts)
e. List important vocabulary terms (write the terms, but explain what they mean to the class during discussion.) (4 pts)
f. Treatment groups (4 pts)
g. Main steps in procedure (8 pts)
h. Highlights of data obtained from study (important data points) and explain why they are important to the conclusion. (4 pts)
i. Main conclusions from paper including if there are further studies needed. (8 pts)
j. Write any questions that you may have or would like to pose to the class.
k. Discuss with the class why this information is important to us. (4 pts) =Total: 40 pts

iii. Quantitative ELISA Lab

Laboratory Procedure

1. Read the patient information below, the remaining procedure, and answer prelab questions.

Patient Information:

- Patient 1: This patient serves as our control. He will still be given a shot, but it will be of 1 x PBS (phosphate buffered saline). There should be no immune response to MMR.
- Patient 2: This patient will only be getting 1 dose of the vaccine that contains only 5 ug of weakened versions of MMR.

- Patient 3: This patient will be getting 2 doses of the vaccine that each contain 5 ug of weakened versions of MMR. These will be given 2 weeks apart.
- Patient 4: This patient will be getting 1 dose of the vaccine that contains 5 ug of weakened versions of MMR AND a 5 ug of ALUM (aluminum salts) as an adjuvant.
- Patient 5: This patient will be getting 1 dose of the vaccine that contains 5 ug of weakened version of MMR AND a 25 ug dose of ALUM (aluminum salts) as an adjuvant.

Pre-Lab Focus Questions: Write the questions and answers in your Legal Scientific Notebook.

a. How does the immune system protect us from disease?
b. How do doctors use the immune response to protect you from disease?
c. What is an example of a disease that attacks the human immune system?
d. What problems can prevent the immune system from working properly?
e. Why is it important to be able to detect antibodies in people who don't appear sick?
f. What does ELISA stand for?
g. Why are enzymes used in this immunoassay?
h. What is an adjuvant (you will need to look this up in your notes if you don't remember)?
i. Predict which patient(s) you think will have the greatest immune response. How much greater do you think it will be from the other patients. Justify your answers.
j. Why do you need to assay positive and negative control samples as well as your samples?
k. If we have positive and negative controls on our ELISA plate, why would we need to have Patient 1 as a negative control also?

2. Follow the 96 well ELISA Plate scheme provided below.

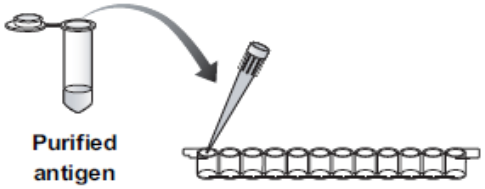
PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	STAND 1000 ng	1:2 STAN	1:4 STAN	1:8 STAN	1:16 STAN	1:32 STAN	1:64 STAN	1:128 STAN	1:256 STAN	1:512 STAN	1:1024 STAN	1:2048 STAN
B	STAND 1000 ng	1:2 STAN	1:4 STAN	1:8 STAN	1:16 STAN	1:32 STAN	1:64 STAN	1:128 STAN	1:256 STAN	1:512 STAN	1:1024 STAN	1:2048 STAN
C	+	P1	P1	P2	P2	P3	P3	P4	P4	P5	P5	x
D	+	P1 1:2	P1 1:2	P2 1:2	P2 1:2	P3 1:2	P3 1:2	P4 1:2	P4 1:2	P5 1:2	P5 1:2	x
E	+	P1 1:4	P1 1:4	P2 1:4	P2 1:4	P3 1:4	P3 1:4	P4 1:4	P4 1:4	P5 1:4	P5 1:4	x
F	-	P1 1:8	P1 1:8	P2 1:8	P2 1:8	P3 1:8	P3 1:8	P4 1:8	P4 1:8	P5 1:8	P5 1:8	x
G	-	P1 1:16	P1 1:16	P2 1:16	P2 1:16	P3 1:16	P3 1:16	P4 1:16	P4 1:16	P5 1:16	P5 1:16	X
H	-	P1 1:32	P1 1:32	P 2 1:32	P2 1:32	P3 1:32	P3 1:32	P4 1:32	P4 1:32	P5 1:32	P5 1:32	x

3. Use a fresh pipet tip to transfer 50 µl of purified MMR antigen (AG) into all wells EXCEPT Column 12 Rows C-H of the 96 well plate.

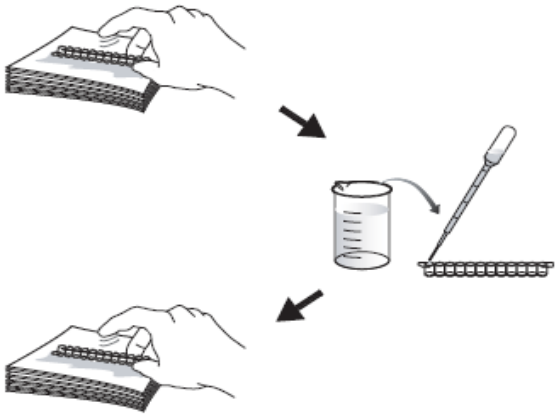
4. Wait 5 minutes for the antigen to bind to the plastic wells.

5. WASH:
   a. Tip the 96 well plate upside down onto the paper towels, and gently tap the plate a few times upside down. Make sure to avoid splashing sample back into wells.



   b. Discard the top paper towel.

   c. Use your transfer pipet to fill each well with wash buffer, taking care not to spill over into neighboring wells. Note: the same transfer pipet is used for all washing steps.



d. Tip the microplate strip upside down onto the paper towels and tap.

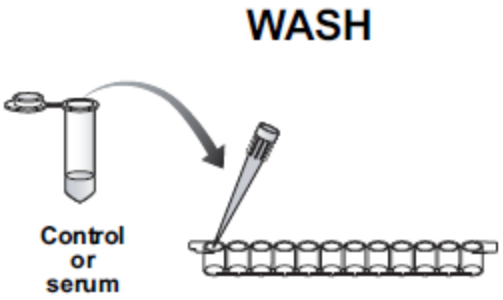
e. Discard the top 2–3 paper towels.

6. Repeat wash step 4.

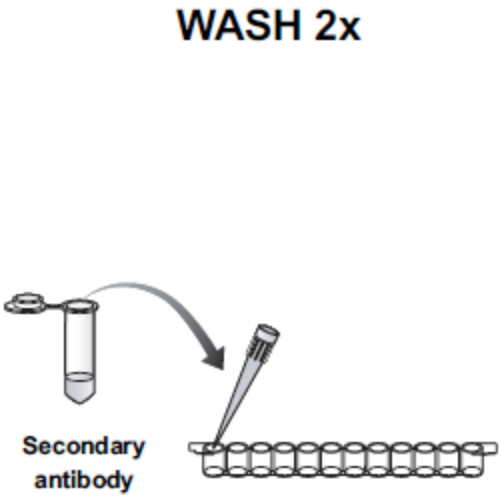
7. Pipet 50 µl of wash buffer into each of your dilution wells before adding samples. Wells: A2-12, B2-12, D2-11, E2-11, F2-11, G2-11, H2-11.

8. Make your serial dilutions of your standard.
   a. Place 100 µl of your standard "STAN" into A1.
   b. Take 50 µl from A1 and place into A2.
   c. Pipet up and down to mix.
   d. Take 50 µl from A2 and place into A3.
   e. Pipet up and down to mix.
   f. Continue this until you get to A12.
   g. Get a fresh tip and repeat for row B.

9. Make serial dilutions of your patient samples.
   a. Place 100 µl of your "P1" sample into C2.
   b. Take 50 µl of C2 and place into D2.
   c. Pipet up and down to mix.
   d. Take 50 µl of D2 and place into E2.
   e. Pipet up and down to mix.
   f. Continue this until you get to H2.
   g. Get fresh tip and repeat "P1" serial dilution in C3 to H3.



10. Get a fresh tip and repeat step 8 for all patient samples "P2" – "P5" in corresponding rows and columns in plate layout above.



11. Use a fresh pipet tip to transfer 50 µl

of the positive control (+) into the three "+" wells.

12. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.

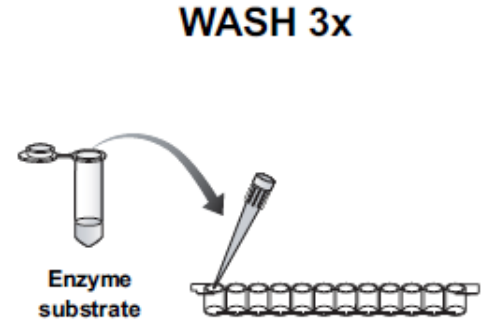
13. Wait 5 minutes for the antibodies to bind to their targets.

14. Wash the unbound primary antibody out of the wells by repeating all of wash step 4 **two** times.

15. Use a fresh pipet tip to transfer 50 µl of secondary antibody (SA) into all wells containing samples.

16. Wait 5 minutes for the antibodies to bind to their targets.

17. Wash the unbound secondary antibody out of the wells by repeating wash step 4 **three** times.



18. Use a fresh pipet tip to transfer 50 µl of enzyme substrate (SUB) into all wells containing samples.

19. Wait 5 minutes. Observe results and take picture with the ENZO ELISA Plate Reader application on your cell phone.
    a. Open the ENZO ELISA Plate Reader application
    b. Click the camera symbol in the bottom left hand corner.
    c. Hold your phone 18 inches about the plate, make sure your plate is in the center of the screen (you can move the black lines later…ignore them for now), and ensure that your plate is square in the camera's view. If it is crooked it will NOT analyze your plate correctly.
    d. Press the capture button.
    e. Click use photo if it is good, if it is not hit delete and retake photo.
    f. Enter photo name.
    g. Exit Program.

iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

1. Obtain patient serum sample.
2. Sort and identify cells by cell markers
3. Classify the cells
4. Record raw cell counts in the Results of Immunophenotyping Table on HIV/AIDS Testing Form
5. Calculate WBC percentages and compare results to reference ranges (make note of any that fall out of reference range)
6. Classify and count CD4 and CD8 cells
7. Record raw cell counts in the HIV/AIDS Specific Results table on the HIV/AIDS Testing Form
8. Calculate CD4%, CD8%, and CD4:CD8 ratio.
9. Compare patient results to given reference ranges and draw a conclusion about AIDS diagnosis.
10. Complete the Flow Cytometry Raw Data Form by making ALL possible dot plots for the 4 parameters listed for 2 different cell types using the same Immunophenotyping Key as a guide.
11. Turn both forms into teacher.

v. Antibiotic Resistance

A. <u>Pre-Lab Questions:</u>

1. Why do organisms like MRSA develop antibiotic resistance?
2. Some scientists argue that when studying antibiotic resistance, it is more important to study the historical sequence of events that led to the acquisition of the gene/trait. Other scientists argue that

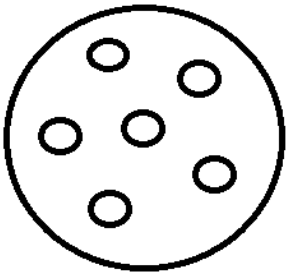
it is more important to study the current function of the gene/trait that provides the resistance? In reality, both aspects are important. Give one example of what can be learned about the resistant organism and/or gene from each scenario.

3. Besides antibiotics, list 3 other common substances that have antimicrobial properties.

B. Procedure:

SAFETY: Wear goggles and gloves at all times. Long hair should be tied back to ensure it does not fall into the flame. Caution should be taken with the flame, bleach, ethanol, and living organism. Although we are using a harmless strain of bacteria you should NOT ingest the living organism.

1. Write title of lab on first blank page in lab notebook and add it to your table of contents.
2. Number your pages
3. Develop a purpose for the lab.
4. Collect all necessary materials from the front of the room and your lab drawer.
5. Clean lab station with 10% bleach and 70% ethanol (Be sure not to get any in your eyes or on your skin. Rinse with water for 15 min if you get in your eye)
6. Light Bunsen burner in lab station.
7. Label bottom of petri dishes with antibiotic names where you plan on placing each corresponding dish.
8. Draw your 3 plates in your lab notebook.
9. Create a data table in your notebook that includes all data that you are required to collect.
10. Use the sterile disposable bulb pipet OR a 100-1000 ul micropipette with sterile tips to add 1 ml of E. coli broth culture to the center of each LB agar plates.
11. Flame sterilize glass plate spreader or inoculating loop and allow to cool
12. Spread the E. coli broth culture evenly around the plate with the sterilized glass plate spreader or wire inoculating loop.
13. Add 1 of each of the 5 antibiotic filter disks and 1 filter disk soaked in sterile DI water to each petri dish in the corresponding labeled spots. Space them evenly as possible to prevent overlap of zones of inhibition.



14. Incubate at 37°C overnight upside down to prevent condensation from building up on the surface of the agar.
15. Observe and measure zones of inhibition for each disk on all 3 plates. Record results and calculate average in data table in your lab notebook.

vi. Ouchterlony Assay Development

1. Using your notes on the Ouchterlony Assay and your knowledge of all other immunological assays that we have done, develop your own protocol for the following scenario.
2. Include the following in your lab notebook as part of your protocol:

a. Title of assay
b. Purpose of assay
c. Background information for anyone doing the lab so they know all the content necessary to understand how to run and analyze the test results.
d. Material and equipment needed (include quantity, volume, concentration, etc.)
e. Advanced preparations needed before the assay is run
f. Experimental parameters (controls, variables, time requirements)
g. A detailed procedure that is able to be reproduced (complete, accurate, and clearly numbered steps)
h. Specify the type of data that will be collected
i. Necessary data tables and reference scales for data collection
j. A written statement of how the technician should write the conclusion of the assay results (what to analyze, interpret, and report to the doctors and patient).

k. Once your protocol is approved, you will run the assay, record the results, analyze the data, write your conclusion, and answer the 3 post-lab questions listed below (all recorded in your lab

notebook).

IV. Data Collection

i. Introduction to Immunology Part 1 and Cell Research Project

None

ii. Introduction to Immunology Part 2 and Vaccines Article

None

iii. Quantitative ELISA Lab

1. Copy the Plate Layout below into your scientific notebook. In the wells across the top row make wells A1 and B1 a value of 1000 ng/ml. That is the concentration of antibody in our standard. Cut that concentration in half as you go to the next well across rows A and B until you get to the last column.
2. Based on the known concentrations in your standard dilution. Eyeball the concentrations of the other wells and fill them in the rest of the table below.
3. An antibody titer is the last well of a patient's sample that has a color change that is just above the blank background wells. Circle the antibody titers for your patients. If you don't have a titer because it is still too concentrated, do not circle one.
4. Copy the Plate Layout again in your scientific notebook.
5. Analyze your 96 well plate samples in the ENZO ELISA plate reader application.
6. Open the ELISA Plate reader application
7. Click on the plate in the upper left corner of the application
8. Choose the image of your plate that you would like to analyze and hit "Analyze"

9. Drag the black lines to the corners of the plate. Make sure they are lined up with the edges of the plate perfectly!!! If not, it will NOT analyze your plate correctly.
10. Hit "Use Photo"
11. Choose 595 nm wavelength to read your plate.
12. It says that the results should be e-mailed to you, but they will not be.
13. Copy the absorbance's down in the second Plate Layout Template you copied into your lab notebook.

PLATE LAYOUT- Write absorbance data that corresponds to each well in the spaces.

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

iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

Follow the instructions on the handout to complete the tables given.

v. Antibiotic Resistance

1. Copy the following chart into your lab notebook and fill in the measured values.

2. Calculate the averages.

	Ampicillin zone (cm)	Chloramphen-icol zone (cm)	penicillin zone (cm)	Streptomy-cin zone (cm)	tetracyline zone (cm)	negative zone (cm)
Plate 1						
Plate 2						
Plate 3						
Average						

vi. Ouchterlony Assay Development

You will be assessed on making your own data table that reflects the lab objectives and outcomes.

V. Discussion/Analysis

i. Introduction to Immunology Part 1 and Cell Research Project

1. Students will individually lead class discussions on their assigned immune cell during and after the presentations.
2. Students will work as a class to build an accurate diagram of immune cell interactions.

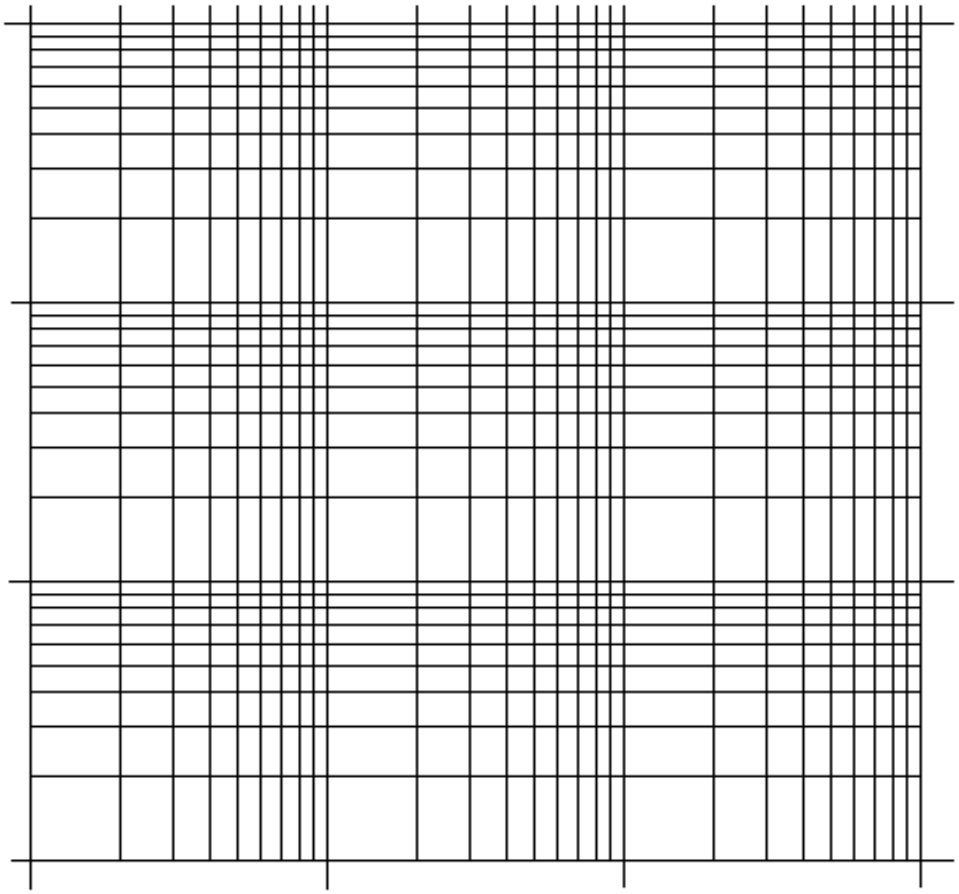
ii. Introduction to Immunology Part 2 and Vaccines Article

1. Students will present their articles and lead class discussion based on findings. Discussion questions will vary based on article topic.

iii. Quantitative ELISA Lab

1. Calculate the average absorbance for each of the sample duplicates. Record in your scientific notebook. (make a table to record these results in to make it easier to analyze)

2. Use the Log scale graph to create a standard curve of your known concentrations of samples in rows A and B. Make sure you take the AVERAGE of the two duplicate well and plot the average concentration. Paste this graph into your lab notebook.



3. Use this graph to determine the actual concentration of antibody in your sample.
4. Copy your absorbance data into the excel document that is posted to the class webpage. It will calculate all your averages and make the graph for you. Print this graph and paste it into your lab notebook. Use that graph and the excel calculated absorbance averages of your samples to determine the concentration of your antibodies in the serum against the vaccine adjuvant. Answer the following questions:
5. Which vaccination method was the most effective?
6. Which vaccination method was the least effective?
7. How did you determine effectiveness of the vaccination from this assay?
8. Why did we do dilutions of the samples?
9. What is a titer?

10. Were we able to get a titer from any of the patients? If so, what were they?

iv. Diagnosis of HIV/AIDS with the use of Flow Cytometry

1. Has your patient progressed from just an HIV infection to AIDS? Support your answer with evidence from the experiment.
2. Is it possible have a low CD4:CD8, but still not have AIDS? How?

v. Antibiotic Resistance

1. Which antibiotics produced the largest and smallest zones of inhibition?
2. What does that mean about the effectiveness of the antibiotic against the pathogen?
3. List the antibiotics in order from greatest effectiveness to least effectiveness based on average zone of inhibition size.
4. What is the purpose of the filter disk that is soaked in sterile DI water in the experiment?
5. Did the varying concentrations of antibiotic on the disks have an effect on the size of the zones of inhibition? Can you accurately draw a conclusion? Justify your answer.
6. What antibiotic(s) would you recommend that the St. Elizabeth's staff treat their "MRSA" outbreak with? If there is more than one, rank them in order from most effective to least effective. Justify your answer.
7. Scientists discovered a new plant in the rainforest that they believe secretes a substance that has beneficial antimicrobial properties. How would you test if the newly discovered plant has antimicrobial properties? Include controls and variables that you would include in the experimental setup.
8. Write a conclusion in your lab notebook that includes RE (results with evidence), PE (possible errors), PA (practical applications), and next steps that could be taken after the experiment.

vi. Ouchterlony Assay Development

1. How likely is it that one Ouchterlony test will give results that lead to the understanding of an organisms' allergic response to antigens?
2. Is the speed of agglutination or precipitation a valuable piece of

data in this experiment? Justify your answer.

3. Setting up an Ouchterlony test may be time consuming. Why not just mix the two solutions together to see if they clump? Suggest an advantage to having the molecules diffuse through and precipitate in the agar.