The Basics of Immunology through Case Studies
Hinkley High School

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This past summer (2014) I had the opportunity to shadow Dr. Harbeck and Dr. Knight at National Jewish Medical Center (NJMC) in the Immunology Department. While spending time in their lab, I observed many of the lab technicians conducting ELISA's, immunodiffusion assays, Kirby-Bauer disc susceptibility assays, and flow cytometry. While at NJMC, I studied the science behind each assay and developed ways to make the assays and the science accessible to high school students. This required finding safe alternatives to many of the chemicals used in the assays while still remaining true to the science behind the assay's purpose. I teach high level International Baccalaureate biology high school classes. My students all plan on continuing to study biology in college, and most desire to work in the medical field after college. As a result, I knew I wanted to present immunology to my students in the most realistic manner possible. This unit was a lot of fun to develop and conduct. My students provided candid feedback concerning the realistic manner of the labs and the methods of teaching the concepts. I hope that you enjoy this unit and that your students find this unit informative, realistic, and interesting.

I. Science Background

Students need a background in basic molecular biology. This immunology unit assumes students understand prokaryotic and eukaryotic cells, cell division, and pathogens. All the assays are thoroughly discussed along with the vocabulary in the provided slideshow. The new vocabulary is presented to the students in the form of videos and graphic organizers. Answer keys are provided for the ease of the teacher.

II. Student Outcomes

- Students will understand the difference between innate and adaptive immunity.
- Students will be able to list and describe the functions of the cells commonly associated with the immune system.
- Students will be able to describe common autoimmune conditions and diseases and the assays used to diagnose them.
- Students will be able to diagnose a "mock" patient with signs and symptoms of a common autoimmune condition or disease.

III. Age and Level

Recommended for advanced/honors students, grades 9-12.

A. International Baccalaureate Assessment Statements

1.1.4 Explain how standard deviation is useful for comparing the means and the spread of data between two or more samples.
1.1.5 Deduce the significance of the difference between two sets of data using calculated values for t and the appropriate tables.
1.1.6 Explain that the existence of a correlation does not establish that there is a causal relationship between two variables.
2.1.4 Compare the relative sizes of molecules, cell membrane thickness, viruses, bacteria, organelles and cells, using the appropriate SI unit.
2.1.5 Calculate the linear magnification of drawings and the actual size of specimens in images of known magnification.
2.2.1 Draw and label a diagram of the ultrastructure of *Escherichia coli* (*E. coli*) as an example of a prokaryote.
2.3.1 Draw and label a diagram of the ultrastructure of a liver cell as an example of an animal cell.
2.4.1 Draw and label a diagram to show the structure of membranes.
2.4.3 List the functions of membrane proteins.
2.5.1 Outline the stages in the cell cycle, including interphase (G1, S, G2), mitosis and cytokinesis.
4.3.1 Define genotype, phenotype, dominant allele, recessive allele, codominant alleles, locus, homozygous, heterozygous, carrier and test cross.
4.3.7 Define sex linkage.

4.4.1 Outline the use of polymerase chain reaction (PCR) to copy and amplify minute quantities of DNA.
4.4.8 Outline a basic technique used for gene transfer involving plasmids, a host cell (bacterium, yeast or other cell), restriction enzymes (endonucleases) and DNA ligase.
5.4.8 Explain two examples of evolution in response to environmental change; one must be antibiotic resistance in bacteria.
6.2.6 State that blood is composed of plasma, erythrocytes, leukocytes (phagocytes and lymphocytes) and platelets.
6.2.7 State that the following are transported by the blood: nutrients, oxygen, carbon dioxide, hormones, antibodies, urea and heat.
6.3.1 Define pathogen.
6.3.2 Explain why antibiotics are effective against bacteria but not against viruses.
6.3.4 Outline how phagocytic leucocytes ingest pathogens in the blood and in body tissues.
6.3.5 Distinguish between antigens and antibodies.
6.3.6 Explain antibody production.
6.3.7 Outline the effects of HIV on the immune system.
7.5.4 State four functions of proteins, giving a named example of each.
8.1.2 Outline the process of glycolysis, including phosphorylation, lysis, oxidation and ATP formation.
8.1.5 Explain oxidative phosphorylation in terms of chemiosmosis.
11.1.2 Outline the principle of challenge and response, clonal selection and memory cells as the basis of immunity.
11.1.3 Define active and passive immunity.
11.1.4 Explain antibody production.
11.1.5 Describe the production of monoclonal antibodies and their use in diagnosis and treatment.
F.1.7 Compare the structure of the cell walls of Gram-positive and Gram-negative Eubacteria.
F.6.1 List six methods by which pathogens are transmitted and gain entry to the body.
F.6.2 Distinguish between intracellular and extracellular bacterial infection using Chlamydia and Streptococcus as examples.

B. Next Generation Science Standards

HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
HS-LS1-3. Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.
HS-LS2-7. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.*

HS-LS2-8. Evaluate the evidence for the role of group behavior on individual and species' chances to survive and reproduce.
HS-LS3-3.Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.
HS-LS4-3. Apply concepts of statistics and probability to support explanations that organisms with an advantageous heritable trait tend to increase in proportion to organisms lacking this trait.
HS-LS4-4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

C. Technical Skills

- Students will learn how to identify gram-positive and gram-negative bacteria.
- Students will learn and practice safety protocols important to immunology.
- Students will learn how to use many different predictive assays to determine possible diseases and conditions; including ELISA's and immunodiffusion.
- Students will learn how to prepare sterile agarose.
- Students will learn how to measure micro amounts of liquid using a micro pipette.

IV. Relevance

The health field is a dynamic and constantly growing field. Any student who is interested in gaining experience in a related health field needs to understand the basics of immunology and how the body's immune system works. This unit will introduce students to innate and adaptive immunity, basic procedures in immunology, assays, common autoimmune conditions, and diseases.

V. Time Requirements

This unit will take approximately 10-15 school days with one class period lasting 60 minutes.

VI. Advance Preparation

A. Order

1. Antibiotic discs mini-set: Carolina Biological #806499 (1 set is enough for 50 experiments)
2. ELISA simulation kit: Carolina Biological #211248 (1 kit is enough for 32 students)

3. Bovine Serum/Anti-Bovine Albumin, Antigen/Antibody set: Carolina Biological #202105 (1 kit is enough for over 100 experiments)
4. Buy case studies book: Case Studies in Immunology, a Clinical Companion, Fifth edition by Raif Geha and Fred Rosen. *Any edition will suffice.

B. Prepare

1. Review and go over all PowerPoint slides and case studies before presenting to the students.
2. Make photocopies of lab procedures and notes for students.
3. Set up lab equipment for ELISA, immunodiffusion, antibiotics resistance lab, and pipetting mini-lab.
4. Ask for faculty and staff volunteers to display symptoms of common conditions and diseases used through this unit to come to a "clinic" in your classroom. Use attached symptoms scenarios for faculty and staff to attend clinic in your classroom for the summative assessment.

C. Materials and Equipment

1. Most materials mentioned in this list are common to a public school classroom. The items that are special to this unit are listed with prices and can all be purchased through Carolina Biological, total cost for special items is: $602.45. This list is for a class of 32 students.

2**. Safety Precautions and Storage:**

Please store ALL chemicals in designated chemical storage area. Keep all chemicals in a cool dry space. Please visit this website for further information regarding chemical storage: http://www.carolina.com/teacher-resources/Interactive/how-to-store-chemicals/tr11069.tr?coId=10856&mCat= . Consult your chemical safety personnel for proper storage and use in your school. Consult your local district and state agencies concerning chemical storage, use and disposal before beginning any of the labs in this unit. While using chemicals safety gloves, goggles and lab coats must be worn by ALL people in the classroom. Disposal of chemicals for the ELISA must be disposed in sinks attached to neutralization tanks. Consult your local, district and state agencies to ensure compliance. The Immunodiffusion and Antibiotic Resistant labs require taping the entire perimeter of the petri dish directly after preparation. DO NOT open the petri-dish again. I place the petri dish in a Ziploc baggie and tape the opening of the baggie as an extra barrier. Once all observation is complete place taped petri dishes in a sink with bleach water for 24 hours. Visit this website for recipe information for bleach water to kill possible harmful pathogens: http://modernsurvivalblog.com/health/disinfectant-bleach-water-ratio/ . Remove tape under the water to allow bleach water into the petri dish, this is to ensure that all harmful growth is killed by the bleach. After 24 hours the petri dishes may be thrown in the trash. Every state has different requirements for possible bacterial growth in public school

classrooms and proper disposal. Please contact your local district and state education agencies before beginning any of the labs.
***Please visit this web-site for further information on waste disposal: http://www.carolina.com/teacher-resources/Interactive/storing-and-disposing-chemicals/tr11068.tr?coId=10856&mCat= .

3. **Antibiotic Resistance lab**:
   Antibiotic discs mini-set ($48.95 Carolina Biological)
   Escherichia coli, Living, K-12 Strain, Tube ($10.95 Carolina Biological)
   Nutrient agar for bacteria growth
   Sterile petri dishes (for agar and discs)
   Heat/stirring plate (for heating up and stirring agar solution)
   Erlenmeyer flasks
   Glass stirring rods
   Heat clamps
   De-ionized water
   Tape
   Inoculating loops (to safely spread bacteria, immediately place in bleach water sink before disposing)
   Incubator ($390.00 Carolina Biological)
   ELISA:
   ELISA simulation kit ($110.00 Carolina Biological)
   Immunodiffusion lab:
   Bovine Serum/Anti-Bovine Albumin, Antigen/Antibody set ($53.50 Carolina Biological)
   Sterile petri dishes
   Straws (for punching holes in agarose)
   Incubator
   Dissecting microscope or magnifying glasses (to observe precipitate lines)
   Powder agar *non-nutrient
   Heat/stirring plate
   Erlenmeyer flasks
   Glass stirring rods
   Heat clamps
   De-ionized water

4. **Micro-pipetting mini-lab**:
   Water
   Test tubes
   Test tube racks
   Food coloring
   Multiple micropipettes in various measurements
   Micropipette tips
   Bacteria cell slide identification:
   Compound microscopes

Slides of gram positive and gram negative bacteria-prepared and sealed (can be purchased through Carolina Biological)
****The micro pipetting mini-lab is not necessary for this unit if time and money are limited. Micro pipettes are approximately $100.00 apiece and can be purchased through bio-rad.com.

VII. Student Prior Knowledge and Skills

A. Knowledge

This unit is for students with a basic understanding of biological concepts especially in relation to the human body. Usually this means that they have taken a general biology course, or this unit could be taught at the end of the school year after they have completed units on human health and physiology and cells. Students need background information on the basics of the immune system, viruses, bacterial infections, antibodies and antigens.

B. Skills

Students should be able to follow lab procedures and safety protocols set forth by the school and district. Students should be able to use basic math computation for many of the labs. Students should have a basic understanding of how to use lab equipment, such as a hot plate, incubator, and microscopes.

C. Preconceptions

Students really struggled with the innate and adaptive immune systems and often confused the two. I found that providing some graphic organizers and interactive assignments for the students enabled them to grasp the differences/similarities. Students have numerous preconceptions about HIV and AIDS. It was helpful to show them a video explaining how HIV is contracted and the biological processes that take place inside a person once HIV is contracted.

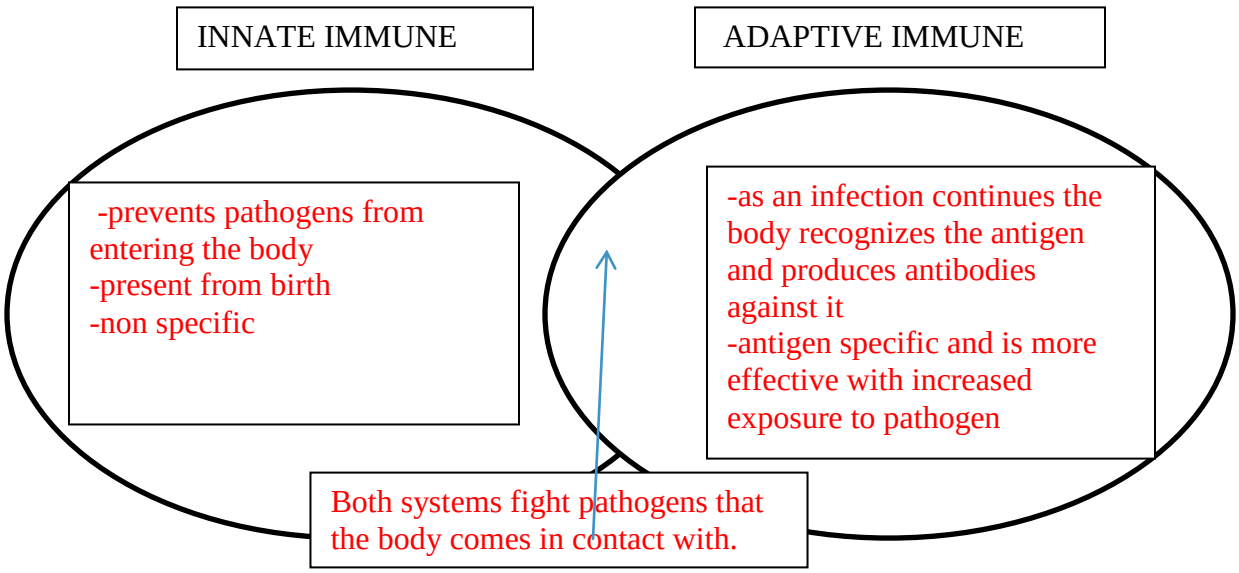
***Consult district and state regulations before teaching HIV as many states have regulations concerning the sensitive topic of HIV.**

VIII. Daily Unit Plans

Day 1: Introduction to the innate and adaptive immune system.
Day 2: Introduction to the key cells involved in the human immune system.

Worksheet: ***Immune System Speed Notes***
Students will use the following resources to fill-out the provided graphic organizer:
http://ib.bioninja.com.au/ (detailed information about required International Baccalaureate biology concepts)
http://www.hhmi.org/biointeractive/cells-immune-system (interactive from Howard Hughes Medical Institute)
https://www.youtube.com/watch?v=WJEc2GDEfz8 (Kids Health video on the immune system, a bit silly, but good explanation)

Immune System Speed Notes
Innate vs. Adaptive
Cells of the Immune System



Cells of the Immune System:

http://textbookofbacteriology.net/cellsindefenses75.jpg

Stem Cell: Cell that can differentiate into other cells.
Lymphoid Stem Cell: Stem cell that specifically differentiates into lymphatic cells.
Lymphocytes: Make up 15% of blood leukocytes.
Natural Killer Cell: Attack and lyse a virus, infected or cancerous cell of the body; make up 5-10% of lymphocytes in the blood.
T-Cell progenitor: May differentiate into a Tc cell or Th cell.
- **Tc cell: Recognize and kill virus infected cells and other altered body cells.**
- **Th cell: Assist cellular and humoral immune systems by secreting cytokines.**

B-Cell progenitor: Differentiates into plasma or memory cells.
- **Memory cell: Provides immunity to future infections from familiar pathogen.**
- **Plasma cell: Secretes antibodies.**

Myeloid Progenitor: Differentiates into granulocytes and dendritic cells.
Granulocytes: Cells containing granules made of mediators.
Neutrophil: Responds rapidly to inflammation. The cells move from blood stream to inflamed tissue where they phagocytize debris and pathogens.
Eosinophil: Migrate from bone marrow to other tissues and kill antibody coated parasites.

Basophil: **Plays a role in inflammation and the allergic response. Found in the blood stream.**
Mast Cell: **Releases histamine and a number of other inflammatory cytokines and mediators. Plays a role in inflammation and the allergic response. Found in tissue.**
Monocyte: **Circulate in the blood stream and migrate to other tissues. Can differentiate into a macrophage or dendritic cell.**

- **Macrophage:** **Phagocytic cell that engulfs and digests microorganisms. Activates T-cells by releasing cytokines.**
- **Dendritic Cell:** **Antigen presenting cells that present antigens to T-cells.**

All of the above are white blood cells (leukocytes).

Days 3 and 4:
Micro pipetting mini-lab (Optional)
This lab was necessary for my students as many had never used a micropipette before. There are an abundance of instructional labs online. I found a few that directly addressed the needs of my students.
https://www.nwabr.org/sites/default/files/IntroToMicropipettingJuly2012.pdf

http://www.usc.edu/org/cosee-west/Jun07Resources/PipetteUsetraining.pdf

Days 5-7:
Case Study #1 HIV
Students will read about a person diagnosed with HIV and complete a mock ELISA.

Book: Case Studies in Immunology, a Clinical Companion, Fifth edition by Raif Geha and Fred Rosen. Case #31 Acquired Immune Deficiency Syndrome (AIDS), pages 187-191
Videos: HIV/AIDS 101 by the Center for Disease Control. Great video about the transmission of
HIV. https://www.youtube.com/watch?v=I_o_wkr7N8A
HIV life cycle: video by Howard Hughes Medical Institute about the life cycle of HIV. https://www.youtube.com/watch?v=odRyv7V8LAE

- Follow the PowerPoint (Case studies slide show) while guiding the students through the reading.
- Using the purchased ELISA (mock) kit from Carolina Biological. Follow the instructions provided for HIV. The kit comes with diagrams, instructions and questions to further the students understanding of how an ELISA works.
- **Note:** Follow all safety procedures. Consult school, district, and state regulations before beginning. Wear goggles, gloves and store chemicals properly. Read statement concerning proper handling and disposal in the Materials section of this publication.

Case Study #1

Human Immunodeficiency Virus (HIV)

Questions to consider while reading:

What sort of questions did the doctor ask?
What observations did the doctor make?

See a figure of HIV structure at:
http://micro.magnet.fsu.edu/cells/viruses/images/hivstructurefigure1.jpg

Background Information

Read the HIV information after the case study. Highlight words/phrases you don't understand.

Now write a 3-5 sentence summary of HIV. Make sure to describe the mechanisms of infection.

See an infographic of HIV infection at:
https://www.aids.gov/images/aids-infographics/what-is-hiv-aids-2.jpg

HIV Questions:

1. A few individuals, mostly hemophiliacs, are know to have been infected with HIV as long as 20 years ago and yet they remain asymptomatic. What factors may contribute to long-term survival with this infection?
2. What is the mechanism of CD4 T-cell depletion in HIV infection?
3. What is the most important know determinant of the progression of HIV infection?

Case Studies in Immunology: A Clinical Companion, Fifth Edition
By: Ralf Geha and Fred Rosen
A Garland Science Publication; Taylor and Francis Group

ELISA

Enzyme Linked Immunosorbent Assay

See a diagram of an ELISA in:
Microbiology: An Introduction,
by Tortora, Funke, and Case, Chapter 18

See an image of an ELISA being performed in a laboratory at:
http://www.pig333.com/3tres3_common/art/pig333/4500/company_news_4500_ELISA-test-at-ISPAH_36628.jpg

Days 8-10:
Case Study #2 MRSA
Students will read about a person with skin rashes and eczema while completing the antibiotic disc lab.

Optional: Observe slides of gram-positive and gram negative bacteria for identification under a microscope.

Book: Case Studies in Immunology, a Clinical Companion, Fifth edition by Raif Geha and Fred Rosen. Case #34 Atopic Dermatitis, pages 207-212.
Video: Atopic Eczema by Eczema Society of Canada; https://www.youtube.com/watch?v=DVRVnvlvUPA
Video: How do antibiotics work? By e-Bug. https://www.youtube.com/watch?v=X1GT2bKgci8

- Before reading: Have students prepare agarose plates according to purchased agarose instructions, swab with bacteria and place antibiotic discs on plate.
- Watch video that shows streaking technique and placement of antibiotic discs. https://www.youtube.com/watch?v=O5NwOGazOAA
- Watch video that shows how to measure the zone of inhibition https://www.youtube.com/watch?v=LzmEwpL2_zI
- Tape the entire perimeter of the petri dishes to enclose them, after streaking bacteria and placing antibiotic discs on plate. I also place the petri dishes in a Ziploc baggy as an additional barrier and tape the opening of the Ziploc baggy. The students are not allowed to take the petri dish out of the Ziploc baggy.
- Place the petri dishes in the incubator. ***Ensure the petri dishes are not incubating at a temperature close to human temperature (98.6F); this is also a precaution to hopefully prevent bacteria from growing that could potentially harm humans.
- Follow the Safety Precautions and Storage procedures above for disposal of the petri dishes when done with the lab.
- **Note:** Follow all safety procedures. Consult school, district, and state regulations before beginning. Wear goggles, gloves and store chemicals properly. Read statement concerning proper handling and disposal in the Materials section of this publication.
- Follow the PowerPoint (Case studies slide show) while guiding the students through the reading.

- After the reading, measure the zones of inhibition for the antibiotic discs.

Case Study #2

Atopic Dermatitis

Questions to consider while reading….

- What sort of questions did the doctor ask?
- What observations did the doctor make?

See images of Atopic Dermatitis at:
https://pedclerk.bsd.uchicago.edu/sites/pedclerk.uchicago.edu/files/uploads/atopic-dermatitis-8-2_0.jpg
and
http://riversideonline.com/source/images/image_popup/sn7_atopicdermatitis1.jpg

Background Information

Read the Atopic Dermatitis information after the case study.
Highlight words/phrases you don't understand.

Atopic Dermatitis Summary

Now write a 3-5 sentence summary of Atopic Dermatitis. Make sure to describe the mechanisms of infection.

Atopic Dermatitis Questions

1. Atopic Dermatitis is described as the 'itch that rashes'. If patients are prevented from scratching, no rash occurs. What is the relationship of scratching to the rash?
2. Skin infections with staphylococci and other bacteria exacerbate AD. Can you suggest a possible explanation for this?

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Gram-positive Bacteria

Gram-positive bacteria are a class of bacteria that take up the crystal violet stain used in the Gram staining method of bacterial differentiation. The thick peptidoglycan layer in the cell wall that encases their cell membrane retains the stain, making identification possible.

See images of Gram-negative and Gram-positive bacteria at:
http://water.me.vccs.edu/courses/ENV108/changes/gram.jpg

Gram-negative Bacteria

Gram-negative bacteria cannot retain the violet stain. Their peptidoglycan layer is much thinner and in between an inner cell membrane and a bacterial outer membrane, causing them to take up the counterstain (safranin or fuchsine) and appear red or pink.

See images of Gram-negative and Gram-positive bacteria at:
http://water.me.vccs.edu/courses/ENV108/changes/gram.jpg

Gram-positive and Gram-negative Bacteria

See diagrams of Gram-positive and Gram-negative bacteria cell walls at:
http://upload.wikimedia.org/wikipedia/commons/thumb/5/52/Gram-Cell-wall.svg/2000px-Gram-Cell-wall.svg.png

Days 11-13:
Case Study #3 Hypersensitivity and Allergy

Students will read about people diagnosed with hypersensitivity to peanuts and allergic asthma, while completing the immunodiffusion lab.

Book: Case Studies in Immunology, a Clinical Companion, Fifth edition by Raif Geha and Fred Rosen. Cases #32 Acute Systemic Anaphylaxis and #33 Allergic Asthma, pages 193-205.
Video: How our bodies develop allergies by Super Scienced; https://www.youtube.com/watch?v=I16cXams0_4
Video: Understanding Asthma by NHS choices; https://www.youtube.com/watch?v=7EDo9pUYvPE
Preparation: Have students prepare agarose plates. Punch holes with straws and fill with antigens/antibodies before beginning to read so that reaction has time to take place and ready to observe after the reading.

- Follow the PowerPoint (Case study slide show) while guiding the students through the reading.
- After the reading, have students observe precipitant lines on plates using light box.
- Tape the entire perimeter of the petri dishes after micro-pipetting the antigens and antibodies in the wells.
- Place the petri dishes in the incubator at approximately 72F/22C. Allow to incubate 12 hours minimum. **Be careful incubating overnight, I unplug my incubator before leaving school for the evening according to safety standards at my school.
- **Worksheet**: **Immunodiffusion Worksheet**

https://www.dshs.state.tx.us/lab/serology_id.shtm (website helps with reading precipitant lines for Ouchterlony worksheet)
https://www.youtube.com/watch?v=Fnx5CkGRBEM (Video procedure for Ouchterlony, used to complete Ouchterlony worksheet)
Note: Follow all safety procedures. Consult school, district, and state regulations before beginning. Wear goggles, gloves and store chemicals properly. Read statement concerning proper handling and disposal in the Materials section of this publication.

Case Study #3

Acute Systemic Anaphylaxis

Questions to consider while reading:

- What sort of questions did the doctor ask?
- What observations did the doctor make?

See an image of an allergic reaction to peanut at:
http://www.celebritydiagnosis.com/wp-content/uploads/2014/04/peanut-allergy.jpeg

Acute Systemic Anaphylaxis

- **Read the Acute Systemic Anaphylaxis information after the case study.**
- **Highlight words/phrases you don't understand.**

Now write a 3-5 sentence summary of Acute Systemic Anaphylaxis.

Questions:

1. Why was John first treated with epinephrine in the emergency room?
1. Why was John given a blood test for histamine and the enzyme tryptase?
2. Why was the skin testing for peanuts not done in the hospital immediately after John had recovered, rather than at a later visit?

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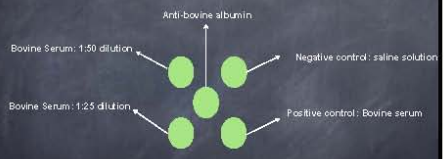
Immunodiffusion (Ouchterlony Assay)

See images of Ouchterlony Assay plates at:
https://www.tk.de/rochelexikon/pics/a28264.000-1_big.gif
and
http://www.snv.jussieu.fr/bmedia/ATP/images/ouchb1.jpg

Set-up for Ouchterlony Plate



Immunodiffusion (Ouchterlony Assay)

Briefly describe in your own words what the assay indicates for:

An antigen and antibody diffuse towards each other in agarose, where the two meet a precipitant line forms. This line indicates sensitivity to the antigen. If a person is not producing the antibodies then their serum will not form a line of precipitate with the antigen.

Write down the procedure for the Ouchterlony Assay:

1. Prepare agarose. (Ensure this is the non-nutrient type)
2. Pour agarose into petri-dishes. Let cool.
3. Use a drinking straw to punch wells into agarose in star design. *see below diagram. Use a sharpie to label the bottom of the petri dish according to instructions.
4. Fill middle well with 10 microliters of antigen.
5. Fill outer wells with patient antibody and positive and negative controls.
6. Place lid on petri dish and wrap perimeter with parafilm. (DO not remove parafilm to observe results as bacteria may grow.)
7. Incubate for 24 hours at approximately 37C.
8. Observe precipitant lines by placing sealed petri dish on light box. (DO not remove parafilm as bacteria might have grown.)
9. To safely dispose of petri dishes place them in a sink of bleach water (one capful of bleach). Underneath the water, remove the parafilm to allow the bleach water to infiltrate the petri dish. Let petri dishes sit in bleach bath for several hours. Make sure to wash hands thoroughly. The petri dishes may be thrown in the garbage after sitting in the bleach bath for several hours.

Describe what the lines of precipitate mean in the two petri dishes at https://www.tk.de/rochelexikon/pics/a28264.000-1_big.gif

-figure I: Since the lines of precipitate cross each other, the reactions are not fully conclusive. This shows that the reactions between A and B affected the reaction of A to AK and B to AK.
-figure II: Since the lines of precipitate do not cross each other, the reactions are conclusive. The antigen AK has reactions with antibodies in outer wells, without the antibodies and controls reacting with each other.

Summative Assessment:
Days 14-15:

Hinkley Wellness Clinic
Faculty and staff at your school will come to your classroom displaying symptoms of common conditions discussed during this unit. Students will be required to prescribe assays and a possible diagnosis based on symptoms. Students will be required to write a paper describing the prescribed assay and diagnosis for their "patient" citing evidence in support of their diagnosis.

- Provide patient symptom sheets for faculty and staff.
- Provide patient questionnaire and patient pain assessment sheets for students to complete while interviewing/diagnosing faculty and staff.
- After students have interviewed/diagnosed a patient, each student should complete the following constructed response based on their interview;
  1. Based on the evidence you recorded, what is your diagnosis for your patient?

  2. Cite ALL evidence that lead you to that diagnosis.

  3. What assay would you recommend the lab conduct to help diagnose your patient?

  4. Write a 3-5 sentence summary of the condition you diagnosed your patient with.

- **Answers:** A student should get the correct condition and symptoms that are listed on the patient symptom sheet. The "patient" should not simply give the student the information on the sheet. A student should obtain all the symptoms and conclude the condition based on their questioning and the information they gather from interviewing the "patient".

Patient Symptom sheet 1

Male or Female

Background: You are a bank manager in downtown Denver. You are slightly overweight but have recently lost 20 pounds without much effort. You equate your weight loss to stress as the bank is currently merging with another bank, so there have been many late night meetings. You have 2 children. The oldest (5 years old) just recently adopted a kitten. While playing with the kitten, it scratched you. Usually a small scratch is no big deal, but the next morning the location of the scratch (hand/arm) was red, swollen, and hot to the touch. The swell on your hand/arm is the size of a baseball. The location of the scratch seems to be infected. This is the reason for your visit to the clinic. You assume that the kitten had some bacteria underneath its claws that gave you an infection when it scratched you. You are at the clinic to get some antibiotics.

Back in college (8 years ago) you engaged in some crazy behavior as college students sometimes do. You faintly remember shooting up (needle) some illicit drugs at a party one night while you were rather drunk. You are not sure if the needle was used previously. This only happened once.

You are not on any prescription medications.
You are healthy other than being slightly overweight.
There is no family history of allergies, anaphylaxis, or angioedema.
Surgeries: appendectomy 15 years ago

****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: ELISA for HIV and Kirby-Bauer Antibiotic disc assay for the possible bacterial infection due to the cat scratch.

Patient Symptom Sheet 2

Female

Background: You are a teenager at a local high school and heard that Hinkley was having a free clinic today. You recently had unprotected sex with a guy at a party. He was super cute and said he didn't have a condom, so you figured it would be OK just this once. Now, your menstrual cycle is 8 days late, and you are afraid you're pregnant. You are here to get a pregnancy test. Absolutely NO ONE knows what you did at the party, (except the guy of course), so you beg the doctor to not tell your parents. The guy you had sex with had already graduated from high school and stated that he has been in college for 3 years now. You have heard gossip that he does sleep around often, but that is gossip so who knows how true it is.

You are not taking any prescription medications.
You do not drink or use drugs.
Your family has a history of heart disease and breast cancer.
You have not had any surgeries.
****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: ELISA for HIV

Patient Symptom Sheet 3

Male or Female

Background: Several months ago while driving home from work, you came upon a person that had just been hit by a car while crossing the street. It was dark outside, and they seemed to be badly hurt. They had a compound fracture on their left leg, and they were losing a lot of blood. You put your hand over the opening to try and stop the bleeding while calling 911 with the other hand. You did not have a first aid kit with you but you vaguely remember first aid training from a few years ago. When the paramedics arrived, they thanked you for your help and helped you clean up the pedestrian's blood that you had all over your hand and arm. The paramedics took down your information as is protocol, since you were the first person at the scene.

One month later, the paramedics called you to let you know that the pedestrian who you helped that night has been diagnosed with HIV. The paramedics suggested that you should also be tested based on your exposure to the pedestrian's blood.

You are extremely healthy-in fact you run several marathons a year.
Age: 35
Occupation: Construction-at new Anschutz Medical Complex
Marital status: single no kids
You have a steady partner whom you have been with for 6 years
Surgeries: tonsillectomy 20 years ago
You do not smoke, do drugs, you drink socially once a week
You are allergic to bees and carry an epi- pen
Family History: heart disease, allergies to various things

****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: ELISA for HIV

Patient Symptom sheet 4

Male or Female

Background: Yesterday, you attended a friend's backyard wedding. The reception was potluck style, and all the guests brought dishes to share. You tried a little bit of everything. You are a rather adventurous person, culinary speaking. This morning, you woke up to a swollen tongue and lips. Your voice is kind of raspy, and it feels as though there is something lodged in your throat.

You are married-no kids
Age: 24
Occupation: teacher
Surgeries: left leg ACL repair in high school- you injured it while playing on the varsity basketball team
Health: You are very healthy-normal weight
You are not taking any prescription medications
Allergies: none
Family History: no allergies, anaphylaxis, or angioedema

****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: Ouchterlony Assay for hypersensitivity to food product

Patient Symptom sheet 5

Male or Female

Background: You are a high school student at Hinkley. Today, during lunch, one of your friends brought in some food that their family had made the night before. You weren't really sure what it was, but it tasted amazing! It was a spicy pork thing wrapped in tortillas. Now, your lips and tongue are swollen. You have red spots all over your arms that itch. You have some extreme stomach cramps, and you are having problems taking deep breaths. You are getting really anxious and scared.

Age: 16
You are allergic to citrus scented products: shampoo, detergent and lotion.
No surgeries
Not taking any prescription medications
Not sexually active
Don't drink, do drugs, or smoke
Family History: no allergies, angioedema or anaphylaxis

****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: Ouchterlony Assay for hypersensitivity to food products

Patient Symptom sheet 6

Male or Female

Background: You have been suffering from stomach cramps for several weeks, which resulted in urgent trips to the bathroom. The stomach cramps seem to occur sporadically, with no known predictability. Currently, there does not seem to be any relationship to meal times. Your partner recently took a cooking class and has been learning how to make some amazing Italian dishes.

Age: 45
Married-4 children
Occupation: Environmental Lawyer
Health: Overweight-but recently lost 8 pounds without effort, high cholesterol
You used to smoke cigarettes but quit 3 years ago
You drink socially and do not use illicit drugs
You take prescription medication for high cholesterol
Surgeries: appendectomy 8 years ago
Allergies: none
Family History: heart disease. No allergies, angioedema, anaphylaxis

****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: Ouchterlony Assay for sensitivity to food products

Patient Symptom sheet 7

Male or Female

Background: You have chronic urticaria. It seems to get worse with sunlight, scented lotions, and detergent. You have been under extreme stress at work lately and have forgotten to take your medications to control the Urticaria. As a result, the rash seems to be significantly worse than usual. You have been itching and itching, (even though you know you shouldn't). Now, the Urticaria seems to be really red, swollen and hot to the touch. The urticaria has developed some scabs due to your constant scratching; underneath the scabs that you scratched white pus often appears. You are visiting the clinic, because the urticaria seems to be worse. Often when this happens, your doctor gives you some prescription steroids, but you could not get an appointment with your regular doctor.

Married-1 child
Your spouse and child also have chronic urticaria
Medications: prescription to control chronic urticaria
Surgeries: none
Health: allergic to milk
Family History: various allergies

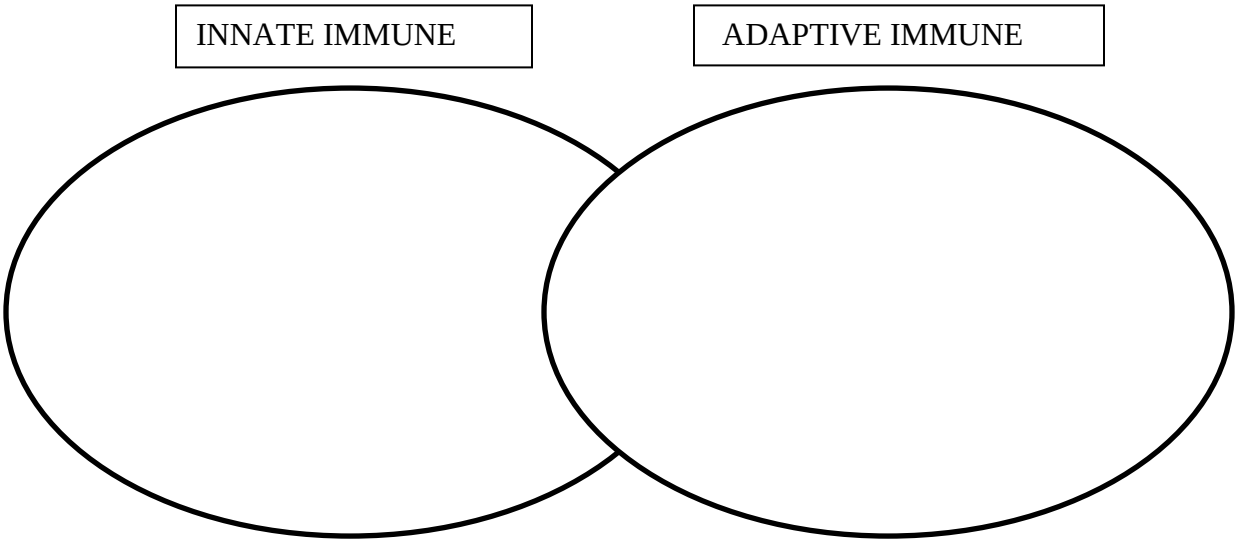
****If the doctor asks you a question that has not been addressed on this symptom sheet feel free to make up an answer that would be consistent with this patient's scenario.

Do not share with student the following information:

Suggested Assay: Kirby-Bauer Antibiotic disc Assay to determine which antibiotic will be most effective in treating the possible bacterial infection on the skin

IX. Student Worksheets

Immune System Speed Notes
Innate vs. Adaptive
Cells of the Immune System



Cells of the Immune System:

http://textbookofbacteriology.net/cellsindefenses75.jpg

Stem Cell:
Lymphoid Stem Cell:

Lymphocytes:

Natural Killer Cell:

T-Cell progenitor:

Tc cell:

Th cell:

B-Cell progenitor:

Memory cell:

Plasma cell:

<u>Myeloid Progenitor</u>:

Granulocytes:

Neutrophil:

Eosinophil:

Basophil:

Mast Cell:

Monocyte:

Macrophage:

Dendritic Cell:

Immunodiffusion (Ouchterlony Assay)

Briefly describe in your own words what the assay indicates for:

Write down the procedure for the Ouchterlony Assay:

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

Describe what the lines of precipitate mean in the two petri dishes at https://www.tk.de/rochelexikon/pics/a28264.000-1_big.gif.

General Questions:

1. What were you doing when the symptoms began?
2. Have you had any recent changes in your diet? Any significant weight gain/loss?
3. What have you eaten today?
4. Do you smoke? Drink?
5. How much sleep do you get on an average night?
6. How often do you exercise?
7. Are you sexually active? Do you use protection?
8. Do you have any allergies to anything?
9. Do you have any pets?
10. Do you take any prescription medications?
11. Have you recently changed any personal care products/laundry detergent?
12. What is your family history? Allergies? Cancer?
13. What surgeries have you had?

Pain Assessment Sheet

Name		File #		Date	

Current Complaints

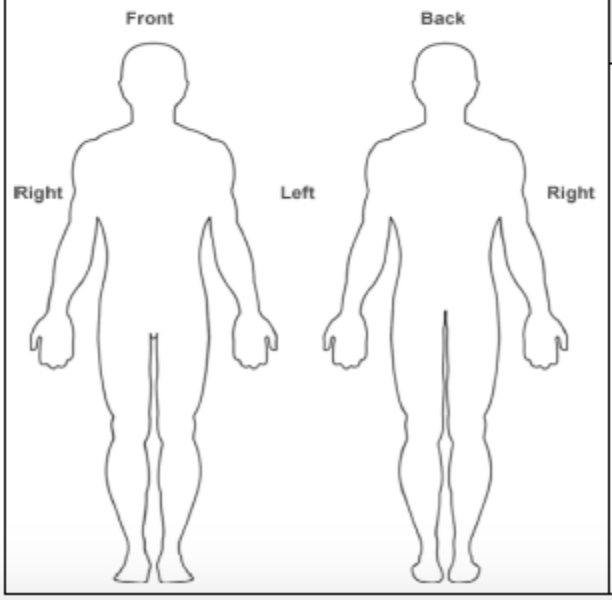
Progression of your current condition since it started	□ Same	□ Improved	□ Worse	□ Other

Does your present condition affect your daily activities at home or in the office? Describe:

Type of pain

□ Sharp	□ Tingling	□ Throbbing	□ Numbness	□ Aching	□ Shooting	□ Dull
□ Burning	□ Cramping	□ Stiffness	□ Swelling	□ Other _____		

Other comments and notes



Describe the areas where you feel pain and provide as much detail as possible. Mark the body outline to indicate location of pain.