Can We Save Them? Using ELISAs to Determine the IL-6 Levels in Septic Patients

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Science Background

Sepsis is an increasing problem in hospitals across the United States. In 2000, there were 621,000 reported cases of sepsis, and that number nearly doubled in 2008 to 1,142,000.[1] From this group, 28% to 50% will die of sepsis.[2] Many pharmaceutical companies have developed potential therapies for septic patients, but none have been successful enough to make it past clinical trials.[3] Thus, much effort has been put into uncovering how sepsis works in order to design a better way to test and treat it.

Sepsis has been defined as the systemic inflammatory response to infection.[4] There are many key players that affect inflammation levels, one family of which are cytokines. Cytokines are small proteins that play a role in cell signaling and are produced by many different immune system cells. Interleukins, which are produced by T cells, can have either a pro- and anti-inflammatory role. Interleukin 6, or IL-6, has been shown to have a strong pro-inflammatory role and is known to be a predictor of mortality.[5] Researchers have also shown that elevated IL-6 levels during sepsis can improve survival when treated with dexamethasone, a common anti-inflammatory steroid.[6]

Thus, if medical professionals are able to test for pro-inflammatory cytokines when they discover that a patient is septic, doctors might have a better chance at selecting appropriate medication faster, leading to fewer sepsis deaths. With great accuracy, the rapid enzyme-linked immunosorbent assay (ELISA) test can detect cytokines. Once blood is collected and blood plasma is isolated, the test takes about 2 hours to get results. Quick lab work such as the rapid ELISA will help save more lives.

What will students do?

Students will use a variety of laboratory skills to perform a rapid ELISA. They will create a standard curve and test patient samples at the same time. They will then have to use analytical and technological skills to process photographs of their finished ELISA to determine if their patients are “sick” enough to receive the medication.

Placement

This unit will be placed after the nervous system and circulatory system units in a full-year Anatomy and Physiology course. Students will have already been exposed to the ideas that cells make molecules to signal to one another (neurotransmitters from neurons) and that cells within the blood circulate quickly throughout the body.

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[1] NCHS Data Brief No. 62 June 2011 - Inpatient Care for Septicemia or Sepsis: A Challenge for Patients and Hospitals

[2] Wood KA, Angus DC. Pharmacoeconomic implications of new therapies in sepsis. *PharmacoEconomics*. 2004;22(14):895-906.

[3] McKenna,M.Researchers Struggle to Develop New Treatments for Sepsis. *Sci Amer*. 19 Mar 13.

[4] Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM Consensus Conference on sepsis and organ failure. *Chest* 1992;101:1481-1483.

[5] Remick, DA, et. al. Six at Six:IL-6 Measured 6H After the Initiation of Sepsis Predicts Mortality Over 3 Days. *Shock*. 2002; 17(6);463-467.

[6] Osuchowski, MF, et al.Stratification is the key: Inflammatory biomarkers accurately direct immunomodulatory therapy in experimental sepsis. *Crit Care Med*. 2009; 37(5) 1567-73.

Student Outcomes

- Students will be able to analyze a case study and design an experiment to test their hypothesis.
- Students will be able to identify the functions of both the innate and adaptive cells of the immune system.
- Students will be able to summarize a journal article on cytokines.
- Students will be able to summarize an article on sepsis, and make suggestions for future research.
- Students will be able to create a standard curve of IL-6 samples.
- Students will be able to evaluate if their samples contain IL-6 by comparing to the standard curve.
- Students will be able to write a formal lab report, and analyze a classmate's report in a peer edit.

Learning Objectives

AP Biology Standards Addressed:

2.C.1. Organisms use feedback mechanisms to maintain their internal environments and respond to external environmental changes.

2.D.3. Biological systems are affected by disruptions to their dynamic homeostasis.

A. Disruption at the molecular and cellular level affects the health of the organism.

2.D.4. Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.

B. Mammals use specific immune responses triggered by natural or artificial agents. (1-6)

3.B.2. A variety of intercellular and intracellular signal transmissions mediate gene expression.

A. Signal transmission within and between cells mediates gene expression (cytokines)

3.D.2. Cells communicate with each other by direct contact with other cells or from a distance via chemical signaling.

A. Cells communicate by cell-cell contact (APCs → T cells)

Next Generation Science Standards Addressed:

• 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. (HS-LS1-3)

• Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS1-1)

• 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. (HSLS1-2)
• 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. (HS-LS1-3)
• Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects. (HS-LS4-2), (HS-LS4-4), (HS-LS4-5)

Daily Unit Plans (45-minute periods)

This unit is intended to be completed in about ten days for 45-minute class periods. Feel free to add/remove lessons as time or resources allow.

Day 1: Introduction to Immunology

Lesson Roadmap:

1. Teacher will assign students into groups of 4. (2 minutes)
2. Teacher will present Part 1 of the case study (project slides on board), and allow groups to discuss the case. (8 minutes)
3. Student groups will present their ideas. (5 minutes)
4. Teacher will present Part 2 of the case study (project slides on board), and allow groups to discuss the case. (8 minutes)
5. Student groups will present their ideas. (5 minutes)
6. Teacher will present Part 3 of the case study (project slides on board), and allow groups to discuss the case. (8 minutes)
7. Student groups will present their ideas. (5 minutes)
8. Teacher will reveal the outcome of the actual case studies and research. (2 minutes)

Day 2: Innate Defenses and Innate Cells of the Immune System

Lesson Roadmap:

1. Students will brainstorm parts of the body that are involved in immune system defenses. (5 minutes)
2. Teacher will lead the class in a discussion of innate defenses. (15 minutes)
3. Teacher will ask the class:
   What happens when pathogens slip by these innate defenses?
   What is the next step your body takes? (2 minutes)
4. Teacher will lead the class is a discussion of innate immune system cells. (23 minutes)

Day 3: Cellular/Specific Immunity

Lesson Roadmap:

1. Students will brainstorm 3 things that they know about T cells, B cells, and antibodies.
2. Teacher will lead the class in a discussion of T cells, B cells, and antibodies.

Day 4: Cytokines

Lesson Roadmap:

1. Teacher will briefly explain what cytokines are and hand out journal article.

2. Students and teacher will read the introduction to the journal article together, popcorn - style.
3. Students will break into groups of 3 and will be assigned one section of the article to summarize.
4. Students will present their summarizations round-robin style to the class. All students will complete a chart on cytokines during this section.
5. Students will research one cytokine used therapeutically in patients for homework.

Day 5: Sepsis

Lesson Roadmap:

1. Teacher will briefly lead the class in discussion of the previous night's homework.
2. Teacher will ask the class:
   What happens when wounds don't heal?
   Or when a surgeon mistakenly cuts an incorrect organ and doesn't suture it?
3. Teacher will briefly lead the class in a discussion on sepsis.
4. Students will read an article on sepsis.

Day 6: Online ELISA lab

1. Teacher will hand out the Student Guide for online ELISA lab.
2. Students will log into their computers and begin working on ELISA lab: http://www.hhmi.org/biointeractive/immunology-virtual-lab
3. Students will hand in their results at the end of class.

Teacher Guide for ELISA Lab

Materials and Equipment Needed for ELISA Lab

- p-20 pipettes and tips
- p-200 pipettes and tips
- p-1000 pipettes and tips
- Cell phone with camera
- Classroom set of computers or tablets with ImageJ (http://imagej.nih.gov/ij/)--free download
- Distilled water
- Eppendorf tubes
- Tube racks
- Refrigerator (4°C)
- Freezer (-20°C)
- 15mL and 50mL plastic tubes
- Tinfoil
- Beakers, flasks, and bottles of varying sizes to store stock solutions
- 5-, 10-, and 25 mL pipettes (glass or plastic), with bulb
- NUNC Maxisorp 96-well plate
- "Blotto" solution (dry milk can be used, but can interact with the antibodies)
- Bovine Serum Albumin (BSA), 2% solution dissolved in PBS
- Tween solution

- Phosphate Buffered Saline solution (PBS)
- Horseradish peroxidase
- Streptavidin
- TMB (3,3',5,5'-tetramethylbenzidine)
- Hydrogen Peroxide, 30% (NOT FOUND IN DRUGSTORES, COMMERCIALLY ONLY)
- Sodium acetate, pH 6
- Sulfuric acid, 1.5M
- Coating IL-6 antibodies (mouse Ab)
- IL-6 standard solution (mouse Ab) (this will also make your "samples")
- Biotinylated IL-6 antibody (mouse Ab)
- Optional, but helpful: orbital shaker, incubator

2 Days before the Lab

Make Dilution Buffer solution, Coating Antibody solution

Recipes for solutions: (* THESE RECIPES ASSUME 15 GROUPS TOTAL, 2 8-well "lanes" each of the ELISA plate)

Dilution buffer:
Reconstitute dry milk as per direction on package with distilled water. Add 10 mL of milk, 5 mL of 2% BSA in PBS, and 50 µL Tween. Bring up the volume to 100mL with PBS solution. Aliquot 5 mL to each student group (use a 15mL tube for each group). Keep in refrigerator until use.

Coating antibody:
Measure 30 mL of PBS in a 50mL tube. Add 30 µL of coating IL-6 antibody to the PBS. Swirl to mix. Keep in refrigerator until use.

For each ELISA plate to be used:
Add 50 µL of the coating antibody solution to each well of the plate. Place in refrigerator overnight.

Day 7: Pipetting Practice/ELISA Sample Prep

1. Teacher will hand out Student Guide for pipetting practice and sample preparation.
2. Students will demonstrate their ability to pipet based on the worksheet.
3. Students will complete the sample preparation.
4. Teacher will hand out the protocol for the ELISA lab and ask students to read and answer the questions for homework.

Teacher Guide for ELISA Lab

1 Day before the Lab:

You will need to make: aliquots of IL-6 Standard, aliquot samples, Biotinylated Antibody solution/Horseradish Peroxidase-SA solution, sodium acetate solution, sulfuric acid solution, and wash buffer solution.

*This prep will take over an hour to complete. A well-trusted student should be enlisted to help label tubes and aliquot samples.

Take diluted IL-6 standard (should be 100 ng/mL) and aliquot 10 µL into an Eppendorf tube for each group. Keep in refrigerator until use.

Aliquot 12 µL of EACH sample (flick tube before aliquoting to mix) into an Eppendorf tube for each group. Keep in refrigerator until use.

Horseradish Peroxidase buffer: Dissolve 0.1 g of Bovine Serum Albumin (BSA) into 100 mL 1X Phosphate Buffered Saline (PBS). Add 0.5 µL of Tween 20. Remove 500 µL, place in Eppendorf tube and add 5 µL of Streptavidin-HRP substrate. Keep in refrigerator until use.

Biotinylated antibody/HRP-SA solution: Combine 5, 928 µL of Dilution Buffer, 12 µL of Biotinylated Antibody, and 60 µL of the Streptavidin-HRP solution made in the last step. Mix and aliquot 500 µL to each student group.

Sodium Acetate: Dissolve 4.1 g sodium acetate anhydrous (MW 82.04) for 500 mL of distilled H2O. Lower the pH to pH=6 with 0.1M Citric Acid (19.21g citric acid dissolved in 1L distilled H2O). Aliquot 12.5 mL to each class. Keep in refrigerator until use.

Sulfuric Acid: Dilute concentrated acid to a 4N solution. Use a glass pipettor and glass storage bottles. When diluting, add acid to the water, NOT water to the acid bottle.

Preparing plates for Desiccation: Dump out the coating antibody from each plate onto a paper towel. Rinse 4 times with 100 µL of Wash Buffer (Recipe below) in each well. Add 150 µL of blocking solution (5% reconstituted milk in distilled H2O--commercially purchased “Blotto” solution is preferable, however) to each well, and incubate for 1 hr. Dump blocking solution out and wash 4 times with 100 µL of wash buffer. Dump the buffer out the 4th time, and TIGHTLY wrap plastic plates with tinfoil, and place in refrigerator overnight.

Wash Buffer: Mix 1 L of 1x PBS with 500 µL Tween 20. Aliquot 50 mL to each group.

Day 8: ELISA Lab

Teacher Guide for ELISA Lab

Day of the Lab:

1. Turn on and set incubator to 37°C.
2. Set up lab benches with two pre-coated well plate strips, samples and pre-made standard curve samples, biotinylated antibody aliquot/HRP, sulfuric acid, and wash buffer.
3. Also, place a tube rack, a p-20 and p-200 micropipette at each station, with pipette tips, and a waste beaker for used pipette tips.
4. Defrost a TMB aliquot at the beginning of each class, keep it in the dark (pocket or drawer) until use. When students are about to wash off biotinylated antibody/HRP-SA solution, mix the 125 µL TMB with 12.5 mL of sodium acetate solution, and 2 µL of 30% hydrogen peroxide. Aliquot 1 mL to each group.
5. Remind students to have their cell phones handy for photograph taking.
6. You might find that 5-10 minutes is not enough time to develop the color reagent from TMB--if so, have the next class take photographs of the previous class' data until the end of the day. The ideal time frame for TMB color development is 20-25 minutes.
7. Students will complete the ELISA lab per the protocol, in groups of two or three. If need be, one student will stay behind to take a picture of their group's finished plate.

Day 9: ELISA Analysis

1. Teacher will hand out ELISA Analysis Guide to students.
2. Students will complete the ELISA analysis, including the image processing using ImageJ software and making a standard curve graph in Microsoft Excel.
3. Students will write a rough draft of their lab reports for homework.

Day 10: Lab Report Peer Edit

1. Students will hand in their rough drafts of their lab reports to the teacher.
2. Teacher will hand back the drafts to a different student, along with a rubric for peer editing.
3. Students will peer edit a classmate's report.
4. The two students will have a quick conversation with each other to give suggestions for improvements and places where the student had done well.

References

Tortora, GJ and B Derrickson. Principles of Anatomy and Physiology. 12th Ed. Hoboken: Wiley. 2008

Jacques, B. "Infectious Disease." Http://sites.tufts.edu/greatdiseases/modules/infectious-diseases /. Tufts University, 1 Jan. 2016. Web. 24 Apr. 2016.

Student Handouts

Name:_____ Per:_____ Date:_____

Immunology Case Study

Scenario 1: What is happening in the wards in this hospital?

Design a study to test this hypothesis. Outline what you would do here. (Remember to think about controls and experimental design!)

Scenario 2: What happened to the doctor?

Design a second study to test this hypothesis.

Name:_____ Per:_____ Date:_____

Cytokine Journal Article

Directions: Read your assigned section of the journal article written by Dr. Charles Dinarello.

Dinarello, CA. Eur. J. Immunol. 2007. 37: S34–45 Historical insights into cytokines

Write down, and look up the definitions of 5 unknown words from your section:

1.
2.
3.
4.
5.

Give a brief summary of your section here (DO NOT WRITE WORDS YOU DO NOT KNOW OR UNDERSTAND. FIND A SYNONYM FIRST.):

_____
_____
_____
_____

Section	Summary

Name:_____ Per:_____ Date:_____

Cytokine Homework

Directions: Choose a cytokine that is used therapeutically (given to patients as medicine).

Which cytokine did you choose?

Which disease is the cytokine used for?

What are some of the known side effects of taking this cytokine?

What is its success rate? (How often does it "cure" patients?)

Is it being researched for any clinical trials?

If you had this disease, would you take this cytokine? Why or why not?

Name:_____ Per:_____ Date:_____

Reading

Read “Researchers Struggle to Develop New Treatments for Sepsis” by M McKenna at Scientific American. 3.19.13

Summarize the article below in the first paragraph. Then, in your second paragraph, suggest what scientists and doctors should research next.

Name:_____ Per:_____ Date:_____

HHMI Virtual Immunology Prelab

Directions: Answer the questions below, using a textbook or internet resources to help. This MUST BE FINISHED by _____at the beginning of class. You will NOT be able to complete the lab if you do not do the following questions:

What is a centrifuge?

What is an antibody?

What is an antigen?

Why do scientists use antibodies to recognize proteins?

What does it mean if someone's blood tests positive for a specific antibody?

What is an ELISA? Explain what it detects in the blood or cell extract.

What is a primary antibody?

What is a secondary antibody?

Why must a secondary antibody be from a different animal than the primary?

What is Horseradish Peroxidase? (HRP)

Why must a scientist incubate their ELISA samples with secondary antibody, and then wash the plate?

What is a spectrophotometer?

Why would a scientist use a spectrophotometer at the end of an ELISA?

What is a serial dilution?

What is Systemic Lupus Erythematosis (SLE)?

What is PBS (phosphate-buffered saline)?

Do you think ELISA is a good method for testing the presence of disease? Why or why not?

Name:_____ Per:_____ Date:_____

HHMI Biointeractive ELISA Lab

Go to this web address:
http://www.hhmi.org/biointeractive/vlabs/immunology/index.html

Or search for HHMI Biointeractive, choose Virtual Labs under the tab, and select "The Immunology Lab"

Then, follow the prompts.

1. What is a common limitation of the ELISA assay?

2. Outline the basic steps of an ELISA.

3. Why are you making a serial dilution for this ELISA? What is its purpose?

4. Explain what positive and negative controls are, and what are the positive and negative controls in this experiment?

5. Notice the ELISA plate has to be washed often. Why is this?

6. What wavelength do you use to observe the color on the plate? Why?

7. Record your results here: (Draw the piece of the microtiter plate that has samples in it, and shade appropriately.)

8. Were you successful at performing your first ELISA? Describe which mistakes you made if you were not (the program provides a checklist at the end).

Name:_____ Per:_____ Date:_____

Pipetting Practice and Setting up Samples/Standard Curve

For this lab, you will be using a p-20 and a p-200 micropipettor. Please observe your instructor's proper use and care of the micropipettor.

1. Go to the goggle box and put on a pair of goggles.
2. Set your p-200 to read: 050. Twist the top dial clockwise or counterclockwise (depending on where the pipettor was set before) until it hits 050.
3. Add a yellow tip to your pipettor, and push to the first stop. Place the tip in the colored water, and gently let the button go, filling the pipette tip with water.
4. Place the pipettor over the note card on the table, and push the button until the second stop, letting the water collect on the note card.

Once you've tried this a few times and feel more comfortable using the equipment, try making one of the solutions below:

Tube #	Red (µL)	Blue(µL)	Buffer (B-µL)	Alcohol (A-µL)	Total Volume
1	50	0	250	0	300
2	75	100	0	125	300
3	0	200	50	50	300

Now, you are ready to make your standard curve and samples! Let's start with the samples.

1. Grab 2 Eppendorf tubes and label them P1 and P2, put your initials on the side of the tube.
2. Put 200 µL of Dilution Buffer (found on the benchtop) in each tube. (Use the p-200!)
3. Then, from the sample stock tube (labeled on benchtop), add 2 µL of sample Patient 1 to the P1 tubes, and add 2 µL of sample Patient 2 in the P2 tube.
4. Grab 6 Eppendorf tubes, and label them A, B, C, D, E, and F. Put your initials on the side of the tube.
5. Put 108 µL of dilution buffer in tube A. Cap tube A.
6. Put 80 µL of dilution buffer in tube B, C, D, E, and F. Cap these tubes.
7. Take 12 µL out of the "STAND" tube and put it in tube A with the buffer.
8. Cap and mix tube A. (Flick it with your fingers.)
9. Take 40 µL out of tube A and put it in tube B. Cap both, and mix both.
10. Take 40 µL out of tube B and put it in tube C. Cap both, and mix both.
11. Take 40 µL out of tube C and put it in tube D. Cap both, and mix both.
12. Take 40 µL out of tube D and put it in tube E. Cap both and mix both.
13. Place all tubes in the tube rack on your bench. These will be used for tomorrow's lab!
14. Remove your goggles and WASH YOUR HANDS!!!!!

Name:_____ Per:_____ Date:_____

ELISA OF INTERLEUKIN-6

Make sure that the following items are on your benchtop:

- samples you made yesterday
- Pipettes, tips, and waste container
- ELISA test strip taped to the benchtop
- Paper towels
- Solutions: Wash buffer, Antibody/HRP solution, TMB solution.

1. Go to the goggle box and put on a pair of goggles and put on an appropriate-sized pair of rubber gloves.
2. Label the edge of your strip with your name, and add 50 of your standard curve to each well:
   - Top well =Tube A
   - 2nd well =Tube B
   - 3rd well = Tube C
   - 4th well = Tube D
   - 5th well = Tube E
   - 6th well = Tube F
   - 7th well = Tube P1
   - 8th well = Tube P2
3. Place your strip in a white plastic carrier in the incubator. Allow it to sit for 15 minutes.
4. Remove strip from the incubator. Dump out samples onto the paper towels. Add 100 µL of wash buffer to each well, tap gently, and dump onto paper towels. Repeat the wash 3 more times.
5. Add 50 µL of Antibody/HRP solution to all wells. Put it in the incubator for 15 minutes.
6. Remove from incubator, dump antibody out onto paper towels. Add 100 µL of wash buffer to each well, tap gently, and dump onto paper towels. Repeat the wash 3 more times.
7. Add 100 µL of TMB solution to each well, cover with foil, and set the timer for 20 minutes.
8. If time remains in the period, take a photograph of your strip once the timer beeps, or before the bell rings.
9. Remove your goggles and gloves, and WASH YOUR HANDS!!!

PS: You may be asked to take a photograph of the previous class' data and upload it to the Google Classroom website. Thank you for helping out!

Name:_____Per:_____ Date:_____

ELISA Analysis by Image J

1. Login to a computer with your login/password.
2. Download your photograph of your test strip to the desktop of the computer.
3. Open ImageJ App.
4. Go to File-->Open and select your photo from the desktop.
5. Go to Plugins-->Analyze-->Color Deconvolution
6. Click ok (for FROM ROI), and make a square over the DARKEST blue circle. Right click to finish the square. Make a second square over the second-darkest circle. Right click to finish square. Make a third square over the PALEST blue circle. Right click to finish the square.
7. The program will now open 3 new pictures, each with only one shade of blue and white. Choose the picture that shows the density of your strip (so, lots of color where you have lots of color, white space where you have white space). ***Sometimes, this app will make a NEGATIVE image, so white space where you had dark blue and dark colors where there was no blue. Either way gets you good data, don't stress!
8. Now, on this new picture, select your ROI (regions of interest). Choose the rectangle or circle tool and draw a region inside the darkest circle. Hit the letter "T". Make a second ROI in the second darkest circle. Hit the letter "T'. Do this for each well of your test strip.
9. As you make ROIs, an ROI manager will appear. Once you've made them all, hit "Measure". A new window will pop up, that says "Results". Copy this entire chart into an Excel or Google Sheets spreadsheet.
10. You will use the "Mean" column for your data.
11. Make a 2 column chart like this:

Concentration	"Mean" number of pixels
10	254
3.3	200
1.1	180
0.4	57
0.1	15
0	0

12. Highlight this chart, and go to Insert → XY scatter (select the graph that connects with lines). Excel has now made you a graph!
13. Add axis labels, a chart title, and tweak anything else on there to make it look pretty.
14. HIT SAVE!!!!! And share it with me via Google Classroom site.

Name:_____ Per:_____ Date:_____

Anatomy and Physiology Lab Report

Criteria for Lab Report	Points allotted	Points earned
Problem, heading and format -Name, Date, and period number on the top left of the first page -Sections of the lab report are clearly labeled starting with the Problem (the question your lab is trying to answer) through the conclusion	5	
Abstract - Brief summary of the experiment (5 - 6 sentences) - Includes introduction, hypothesis, methods, hypothesis supported or not	10	
Purpose - 1 to 2 sentences explaining the purpose	5	
Hypothesis & Prediction -If …. then… because… statement - Addresses the problem	5	
Materials -Listed w/ Amounts of each material used	5	
Methodology (procedures) -Specific description (either list of steps or in paragraph form) -Safety precautions -Including how to manipulate data (if statistics are used)	5	
Data Table(s) - In an organized chart/table -Several trials	10	
Graph (computer generated) - Correct type of graph -Title graph and label axis - Correct info on the axis - Answers the questions asked	10	
Discussion -State whether the hypothesis was supported by your experiment or not and WHY -Use evidence (data, observations, calculations, or statistics) to support your statement. -Compare and contrast the results you obtained with the results you expected and why! -Discuss possible sources of error and how they may have affected the results. Be specific.	30	
Conclusion -One to two sentence conclusion - Includes hypothesis	5	
Resources - Resources should be cited in the discussion using MLA format - Resources should also be included at the end of the paper	10	
Total Points **** Please note that this is a FORMAL lab report. Do not use pronouns (I, we, us, me) or slang terminology.	100	

Kudos, Comments, Concerns…

Name:_____ Per:_____ Date:_____

Peer Edit Sheet

1. Read each section of the lab report.
2. As you read, **make corrections** as you see fit. Place a check in the small circle next to the item if the writer has completed the task. **If the writer has not, please provide specific feedback on how they could improve.**

Format

- o 12 point font, if typed
- o One inch margins
- o All sections start with appropriate headings.
- o All sections follow in order of the rubric.

Necessary Components

- o A scientific title (Do you know what the experiment is about?)
- o A clear problem statement with variables identified, if applicable
- o A clear hypothesis in the IF… THEN… format
- o Materials listed
- o Procedure easily followed (could you follow what they wrote and re-do the lab?)
- o Results section in a table and graph if applicable
- o Conclusion containing specific information with support of the data
- o Re-statement of the problem
- o What happened in the experiment?
- o Support using THREE pieces of data
- o WHY the experiment worked out that way
- o Concluding statement
- o Comments on validity
- o Two uncontrollable errors
- o Two improvements

Grammar checklist

- o All sentences begin with a capital letter.
- o Appropriate punctuation is used.
- o Past tense is used. (Ex: An experiment was performed.)
- o Contractions are **not** used (can't vs. cannot, didn't vs. did not, etc.).
- o Sentences are clear and concise (no run-ons).
- o Scientific language is used.