Catalase Enzyme Activity

Enzyme Catalysis Lab
Teacher's Guide

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Teacher Guide

Unit

This lab fits in Chapter 2 (Chemistry of Life) of Freshman Biology with enzyme activity.

Overview

In this lab, students will use yeast as a source of catalase. Students will change the temperature of the substrate, hydrogen peroxide, and measure oxygen production with a gas pressure sensor. As oxygen is produced, gas pressure will increase.

Lesson Plan

This lesson can be completed between 1-4 days, but the full four days are recommended.

Day 1: Introduce enzymes to students (maybe lecture for 15-20 minutes only). Read through the student lab guide with the students.
Day 2: Conduct the lab.
Day 3: Debrief lab results and help students complete the lab graphic organizer

Materials and Preparation

Materials for each group bucket:

1 Lab handout (laminated)
1 LabQuest (fully charged)
1 Gas pressure sensor
Plastic tubing with 2 Leur locks and 1 small black stopper attached
1 Thermometer
1 10ml graduated cylinder
1 Test tube rack
3 18x150mm test tubes
3 Labeled pipets (water, yeast, H2O2)
1 Bottle hydrogen peroxide
4 Styrofoam cups

Advance Preparation

At least 1 day in Advance:

- This lab is pretty easy to set up, but you do need to test it before the students.
- Set up all the group lab materials in baskets for each group (see the Laboratory Sheet for a list of materials).

- The gas pressure sensor boxes have extra materials that are needed for other labs. Please **DO NOT LOOSE THE PARTS NEEDED FOR OTHER LABS!** You will need:
  - The **short** tubing
    - The length of plastic tubing connecting the rubber stopper assemblies to each gas pressure sensor must be the same for all groups. It is best to keep the length of tubing reasonably small to keep the volume of gas in the test tube low. If pressure changes during data collection are too small, you may need to decrease the total gas volume in the system. Shortening the length of tubing used will help to decrease the volume. For this reason, shorter tubing is provided (DO NOT CUT THE TUBING—IT IS NEEDED FOR OTHER EXPERIMENTS!)
  - The two Luer locks (small beige screw on connectors) must be attached to both end of the short tubing. PLEASE DO NOT LOOSE THESE LOCKS!
  - The black stopper
  - All other materials (clamps, big stopper, syringe, etc.) can stay in the box. Please do not give them to the students because they will play with them and loose them.

- IMPORTANT NOTES:
  - You must use **fresh** hydrogen peroxide and yeast every year for this lab to yield decent results. Yeast can be bought at any grocery store (look at the expiration date closely) and hydrogen peroxide can be bought at any pharmacy. If peroxide is kept in the refrigerator, it must be brought to room temperature before the lab.
- Set out several large beakers of water so the water equilibrates to room temperature overnight.
- You must run a trial of this lab to make sure that the yeast suspension is at the right concentration/activity (it will vary depending on the age and quality of the yeast).
- To make yeast suspension, add the following into a Styrofoam cup (**Make a fresh suspension each period just before the passing period.**):
  - 100 ml warm water (37-40C, but no hotter or the yeast will die or enzymes will denature).
  - 1 packet of yeast (approx 7g)
  - 1 packet of sugar (the little packets they give at restaurants) (or make a 2% sugar solution by adding 4 g sugar to 250 ml water, then add a packet of yeast to 100 ml of this solution.)
  - Let the solution sit for 10 minutes before testing.
    - The room temperature reaction with two drops of suspension **should produce around 130 kPa (1.3 atm) in 40-60 seconds.** Pressures above 130 kPA will cause the stoppers to pop off. Pressures above 210 kPA may damage the sensor.
    - Add more warm water, if necessary, to dilute the suspension.
    - If the solution isn't strong enough, have students add more drops. Test the number of drops in advance to ensure you can get results near 130 kPA after 1 minute.
    - add step- have students use paper towel on dropper to wipe off foam and keep off of side

Morning of Lab:

- Using a hot plate, prepare enough hot water for all lab teams. The water should be 50-55C. Warn students to be very careful with the hot water.

Immediately Prior:

- For best results, prepare a fresh yeast suspension approximately 10-20 minutes before use in each class. See instructions above for how to make the yeast suspension.

Variations and Extensions

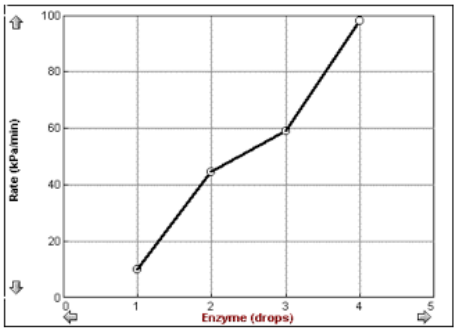
You can modify this lab to test the effect of enzyme concentration, pH, or salinity. Similarly, you can follow up this experiment by having students designing their own experiment to test one of these factors.

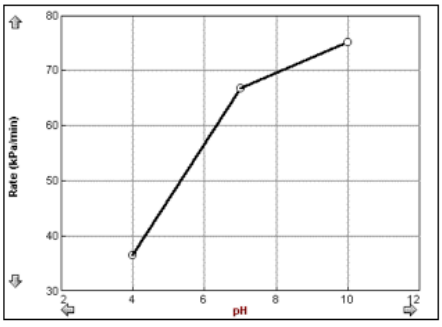
- Enzyme Activity: With 3ml H_2O_2 and 3ml H_2O in each tube, add 1 drop of enzyme suspension. Repeat with 2, 3, and 4 drops.
- pH: Replace the water in the test tubes with buffers (pH 4, 7, 10).
- Salinity: Replace the water in the test tubes with various salt concentrations (very dilute) or have students add drops of 1M NaCl much like they did for testing concentration
- Sugar source: Make the yeast suspensions with various types of sugars (brown sugar, granulated, powdered, glucose, fructose, lactose, etc).
- Enzyme source: Make the enzyme solution with catalase from different sources: beef, chicken, yeast, turnips, bakers' vs. brewers' yeast, etc.
    - To prepare a liver suspension, homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water. You will need to test the suspension before use, as its activity varies greatly depending on its freshness. Dilute the suspension until the experiment produces a pressure of 130 kPa in 40 to 60 seconds. The color of the suspension will be a faint pink. Keep the suspension on ice until used in an experiment.

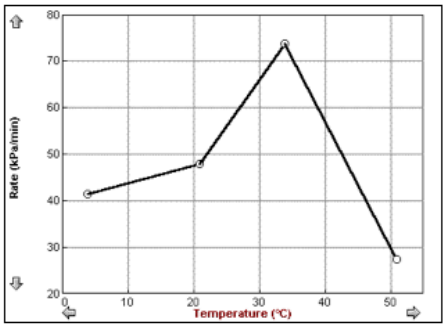
Sample/Key

Sample experimental results for various conditions

Sample Data	
Condition	Slope, or rate (kPa/min)
1 Drop	10.23
2 Drops	44.98
3 Drops	59.36
4 Drops	98.26
4°C	41.43
21°C	48.02
34°C	73.85
51°C	27.55
pH 4	36.57
pH 7	66.86
pH 10	75.27







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Hindsight

After conducting the lab, teachers have these suggestions:

- Students in general biology classes, largely did not understand the concept of slope and rate. Teachers need to make these connections very obvious for students. Make sure students look at m and not b in the data.
- Try to do a lab in advance where students have already done page two of the graphic organizer.
- When students sketch a graph on the lab report graphic organizer, it would be best to have them make a BAR GRAPH of the rates rather than sketch a line graph of all 3 runs. The bar graph helps students focus on the rate of reaction rather than individual data points.
- Suggestion: Assign tasks for each student (i.e. Test Tube holder, Procedure specialist, etc.).
- If students add too much enzyme, the stopper pops off (especially with the hot water). This can affect their slopes.
- Several students were spilling the cups of water and had to start over. To solve this problem, double up the cups and place some sand in the bottom cups to weight them down.
- Don't do the lab on a shortened scheduled (Wednesdays) because they will not finish. Also, explain the lab THE DAY BEFORE so they have the entire period to work the day before. Possibly video the lab demo for the students and have them watch it the night before in order to save class time on explanation.

Catalase Enzyme Activity

Student Guide

Objective
Understand the role of changing environmental conditions (temperature) on enzyme activity

Standards
Biology 1b: Students know enzymes are proteins that catalyze biochemical reactions without altering the reaction equilibrium and the activities of enzymes depend on the temperature, ionic conditions, and the pH of the surroundings.

Introduction
Almost all chemical reactions that occur in living organisms are catalyzed by enzymes. Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. They act as catalysts, substances that reduce activation energy, or the energy it takes to get a reaction started. Enzymes are not destroyed or altered during the process. They are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second. Many factors in a cell's environment, like temperature, pH, concentration and salinity, affect the action of an enzyme. Changes in these conditions may change the shape of the enzyme making it useless, a process called denaturation. In this investigation, you will conduct an experiment to determine the effect of temperature on an enzyme catalyzed reaction.

In every cell of the body, hydrogen peroxide, H_2O_2, is produced as a waste product. However, hydrogen peroxide kills living cells. Although H_2O_2 is toxic to most living organisms, many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2 can be converted to oxygen and water, as follows.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Because your liver is responsible for detoxifying your blood, your liver cells contain a lot of catalase to break down H_2O_2 into harmless H_2O and O_2. Yeast cells also contain catalase, so we will use them as an easy and inexpensive source of catalase in this lab.

When the H_2O_2 encounters the yeast catalase, it bubbles, as the gaseous O_2 escapes. We will measure the amount of O_2 being produced using a gas pressure sensor. The more O_2 that is produced, the greater the pressure in the tube will become. An increase in pressure tells us that the catalase is effective at breaking down the H_2O_2.

A graph of gas pressure over time will be generated by the computer. Your graph may look something like this:

We know from our math classes that the line in this graph has an equation of y = mx + b. For our purposes, we only care about m, the slope of the line. The slope, or steepness of the line, tells us how quickly the gas pressure is increasing. The steeper the line, the greater m will be and the more oxygen is

being produced telling us that the catalase is working well.

But what about the units for this slope? The slope, m, tells us the pressure generated every minute, so the units would be kPa per minute, or kPa/min. Kilopascals, or kPa, are just a unit of pressure; you may have heard of other units of pressure like pounds per square inch (PSI) in car tires or atmospheres (atm) or millimeters of mercury (mmHG) for weather pressure. For example, if m is 0.00376 our rate of gas production is 0.00376 kPa/min. The larger the slope, the more oxygen is generated and the more active the enzyme is at those conditions.

We will compare the slope of the lines at 3 different temperatures: hot, cold, and room temperature. Which temperature do you think will have the highest rate? The lowest?

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Laboratory Sheet

Safety Precautions

Be very careful with the hot water in this experiment.

Lab Team Materials

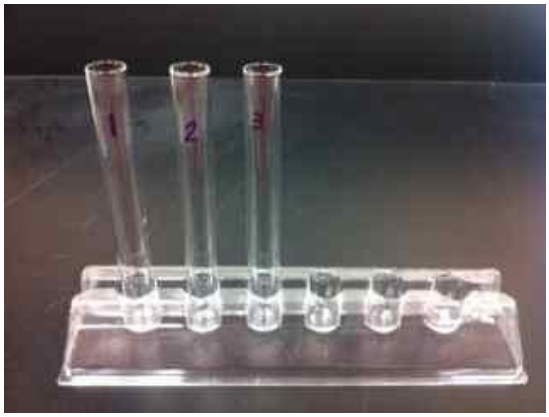
1 LabQuest
1 Gas pressure sensor
1 Tubing set (with rubber stopper and 2 Leur locks)
10 mL graduated cylinder
Tap water
4 Styrofoam cups
1 Bottle FRESH 3% H_2O_2
3 18 x 150mm test tubes
1 Test tube rack
10 mL yeast solution
3 Labeled pipets (Water, H2O2, Yeast)
1 Thermometer

Shared Class Materials

3 water baths (room temp, hot, ice)
Yeast solution

Procedure

1. Place 3 test tubes in a rack and label them 1, 2, 3.



2. Add 3 mL of 3.0% H_2O_2 and 3 mL of tap water to each test tube (Use a plastic pipet/dropper to easily dispense the water and H_2O_2—don't mix up the pipets!).

3. Fill a Styrofoam cup half way with water from the room temperature bath in the front of the room. Place Tube 1 in this beaker. Fill a second Styrofoam cup half way with tap water and ice. Place Tube

2 in this beaker. Fill a third Styrofoam cup half way with hot water from the hot water bath in the front of the room. Place Tube 3 in this beaker.

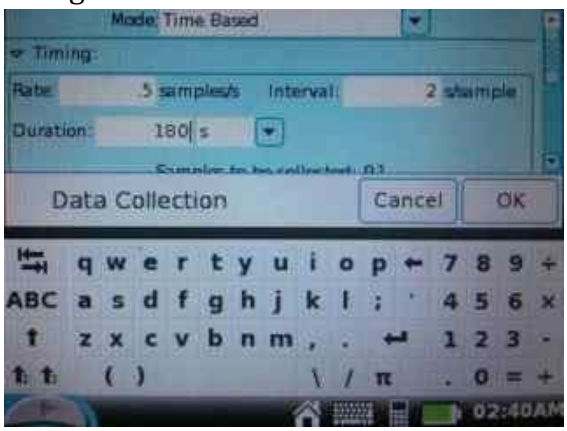
4. Place thermometer in 1 water bath. After the temperature has stabilized and the reading has not changed for 5 seconds, record the temperature in the data table of the Freshman Biology Graphic Organizer. Repeat for the remaining 2 water baths.

5. Leave the tubes for 5 minutes while you complete the next steps.
6. Connect the plastic tubing to the valve on the gas pressure sensor.

7. Connect the sensor to CH1 on the LabQuest.

8. Turn the LabQuest on. On the Meter screen, tap Rate.

9. Change the data-collection rate to 0.5 samples/sec and the duration to 180 seconds. Press OK.

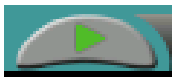


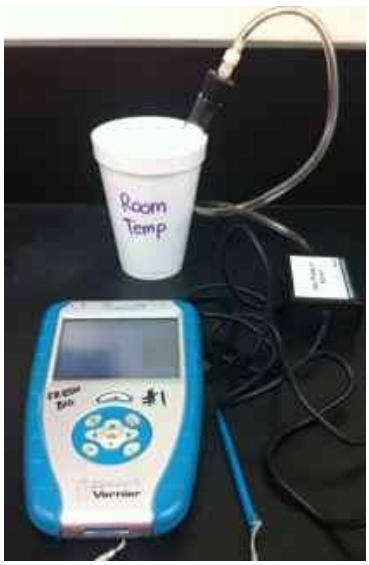
NOTE: Read ahead and be prepared to move on to the next 3 steps QUICKLY.

10. Add 2 drops of yeast enzyme solution to Tube 1 (Do not let the solution stick to the sides of the tube--get the pipet close to the solution, but not in it.).

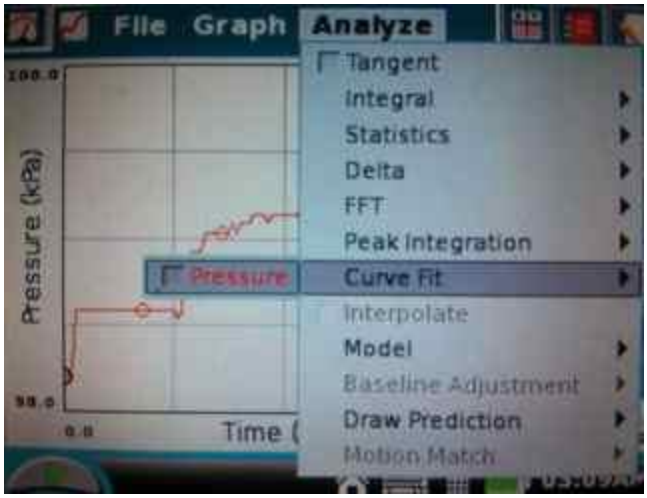
11. Put the stopper on the tube and GENTLY swirl the tube to mix the contents.

12. Start data collection by pressing the green play icon. Data collection will stop after 3 minutes. (Monitor the reading on the screen. If the pressure exceeds 130 kPa, disconnect everything and start over with less enzyme solution.)

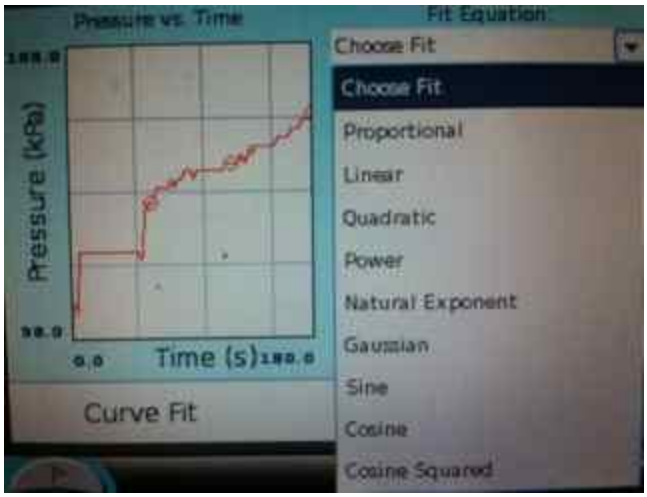




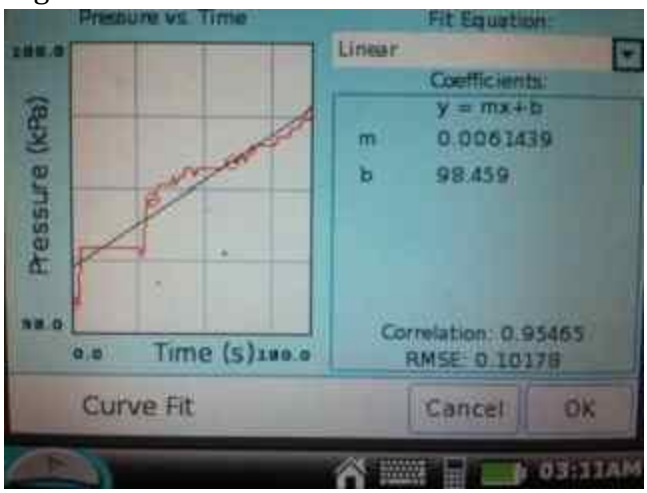
13. Disconnect the stopper from the test tube. Rinse out the test tube and leave it upside down in your rack to dry.
14. Choose Curve Fit from the Analyze menu. Check mark Pressure.



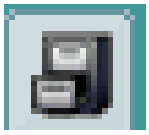
15. Choose Linear Fit to get an equation in the form y=mx+b



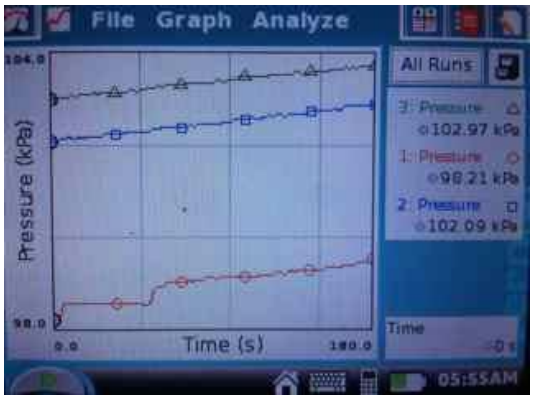
16. Record the slope of the line, m, as the reaction rate in the data table of the Freshman Graphic Organizer. Select OK.



17. Store the data from the first run by tapping the File Cabinet icon.



18. Repeat steps 12-21 for Tubes 2 and 3, replacing the room temp water for the ice water and hot water, respectively.
19. Tap Run 3. Select All Runs. All three runs will now be on the same graph.



20. Rinse out all your test tubes and leave them upside down to dry. Complete both sides of the Graphic Organizer as your lab report.

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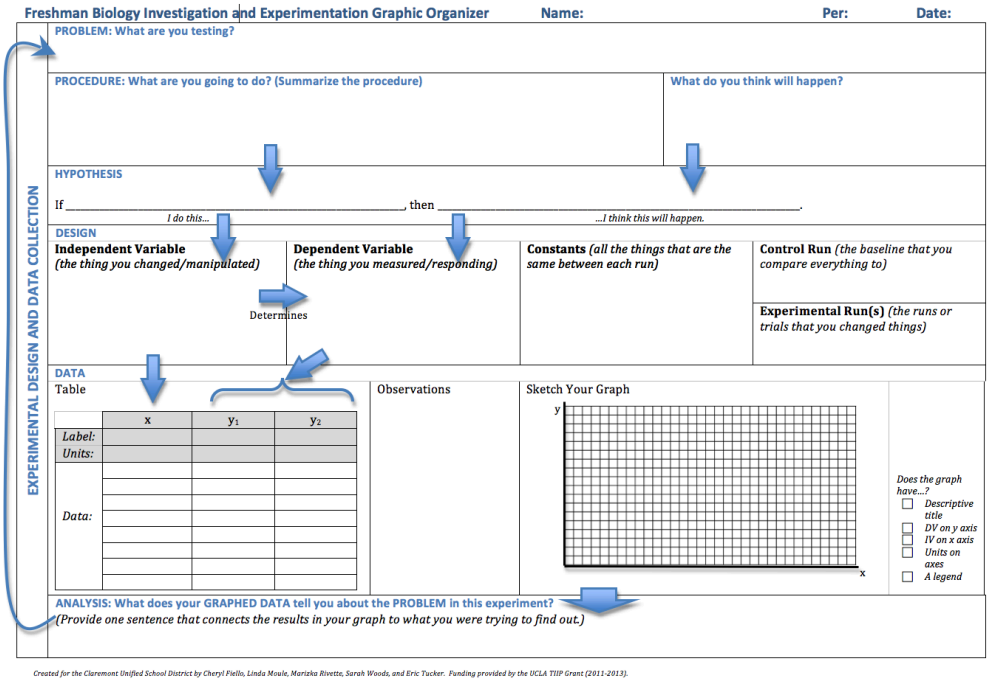
Student Lab Sheet

Use pages 1 and 2 of the [Freshman Biology Graphic Organizer.](#) The second page is the most essential for this lab.



Freshman Biology Investigation and Experimentation Graphic Organizer **Name:** **Per:** **Date:**

PROBLEM: What are you testing?

PROCEDURE: What are you going to do? (Summarize the procedure) | **What do you think will happen?**

HYPOTHESIS

If _____ (*I do this...*), then _____ (*...I think this will happen.*).

DESIGN

Independent Variable *(the thing you changed/manipulated)* | **Dependent Variable** *(the thing you measured/responding)* | **Constants** *(all the things that are the same between each run)* | **Control Run** *(the baseline that you compare everything to)*

Determines

Experimental Run(s) *(the runs or trials that you changed things)*

DATA

Table

	x	y_1	y_2
Label:			
Units:			
Data:			

Observations

Sketch Your Graph

y

x

Does the graph have...?
- ☐ *Descriptive title*
- ☐ *DV on y axis*
- ☐ *IV on x axis*
- ☐ *Units on axes*
- ☐ *A legend*

ANALYSIS: What does your GRAPHED DATA tell you about the PROBLEM in this experiment?
(Provide one sentence that connects the results in your graph to what you were trying to find out.)

EXPERIMENTAL DESIGN AND DATA COLLECTION

Created for the Claremont Unified School District by Cheryl Fiello, Linda Moule, Marizka Rivette, Sarah Woods, and Eric Tucker. Funding provided by the UCLA TIIP Grant (2011-2013).

INTRODUCTION

What were you trying to find out?

What did you do to find it?

What did you think would happen?

THESIS STATEMENT

What is your ONE most important piece of data you collected? | Why did you do this lab? | How good are your results?

BODY

	Paragraph 1	*Paragraph 2*	*Paragraph 3*
Concrete Statements 1	Provide a statement of the data trend.	List the major concepts or vocabulary from class that are in this lab. Circle the most important item from this list.	What are the sources of error in this experiment?
Concrete Statements 2	What does the data tell us about the problem?	**Explain how the circled term applies to THIS lab.**	How could you fix those errors?
Concrete Statements 3	Was your hypothesis right? Why did you think that would happen?	What standard, key concept, and/or objective does this lab address?	If we did this lab again, what other independent variables could we test?

CONCLUSION

Restate your thesis in different words.

What is so important about this lab to our learning?

ANALYSIS AND CONCLUSION