

Liver Lab Procedures

Read all directions carefully. If you don't understand ask Ms. Leyda.

Lab Overview and Checklist:

Day 1: Prepare Liver Samples:

- A: 3 Raw liver pieces in 6 mL water in zip lock bag
- B: 3 Boiled liver pieces in 6 mL water in zip lock bag
- C: 3 Lemon liver pieces in 6 mL lemon juice in zip lock bag
- D: 3 Salt liver pieces in 6 mL of salt water.
- E: 3 Cold (Raw) liver pieces in zip lock bag Day 2 we will ice it)

Day 2: Test level of peroxidase by adding hydrogen peroxide to each of the liver samples.

- Take one of each liver sample A, B, C, D and E, put it into the correctly labeled test tube.
- Add exactly 2 mL of hydrogen peroxide:
 - o **Before adding:** have thermometer in test tube before you add the hydrogen peroxide.
 - o **During reaction:** measure how high bubbles reach in the tube with the ruler in cm
 - o **Stop timer** at end of large part of reaction, or after 2 min if it lasts that long.
 - o **Take temperature** of highest temperature reached during reaction

Detailed Lab Procedure: Day 1

Materials: Checklist to make sure you have all materials.

- | | |
|---|--|
| <input type="checkbox"/> Beaker with water and a pipet | <input type="checkbox"/> 1 tray (to use for a cutting board and holding materials) |
| <input type="checkbox"/> Salt in a cup with spoon | <input type="checkbox"/> ¼-½ of a lemon |
| <input type="checkbox"/> Hot water bath | <input type="checkbox"/> 1 Piece of liver (you will cut up) |
| <input type="checkbox"/> Test Tube rack with 5 Test Tubes | <input type="checkbox"/> 5 plastic zip lock bags |
| <input type="checkbox"/> 1 permanent pen (Sharpie) | <input type="checkbox"/> Test Tube Brush for Cleaning Test Tube |
| <input type="checkbox"/> 1 forceps | <input type="checkbox"/> thermometer (Day 2 only) |
| <input type="checkbox"/> 1 dissection scissors | <input type="checkbox"/> Timer (Day 2 only) |
| <input type="checkbox"/> 1 funnel (to collect lemon juice) | <input type="checkbox"/> 1 ruler (Day 2 only) |
| <input type="checkbox"/> 1 sink bucket for cleaning (Day 2) | <input type="checkbox"/> Hydrogen Peroxide (Day 2 only) |
| <input type="checkbox"/> 2- 10mL Graduated Cylinders | <input type="checkbox"/> 4 weigh boats (Day 2) |
| <input type="checkbox"/> Electric Balance | |

Day 1: Laboratory Procedures: Liver Enzyme Lab

Step 1: Label all 5 plastic bags with Method ___ Per ___ Lab ___

Step 2: Cut your piece of liver into 5 equal size pieces. Each of these pieces of liver will be prepared using one of the Methods below (A, B, C, D, or E). Because all GOOD scientific experiments do a minimum of 3 trials, you must take each of the 5 pieces of liver that you have and cut them into 3 equally sized pieces. This way you can do 3 trials with Method A, 3 trials with method B, etc. for all 5 different methods.

Step 3: Divide up the tasks. First priority is to get 3 pieces of liver in a test tube with 6mL of water and get them boiling. Have one group do Method 'A and E', and B, and the other half of your group do Method C and D.

Step 3:

| | |
|---|---|
| <u>Method A: Raw Liver</u> | <u>Method B: Boiled Liver</u> |
| <p>Using plastic bag:</p> <ul style="list-style-type: none"><input type="checkbox"/> Label the bag: “Method A: Raw” and write your period number & lab station number<input type="checkbox"/> Place 3 equally sized pieces in the bag.<input type="checkbox"/> Using a graduated cylinder, measure out 6 mL of water. Add this water to the bag.<input type="checkbox"/> Seal the bag. (Make sure to squeeze the air out of the bag first). | <p>Using your Bunsen Burner:</p> <p>Using a test tube:</p> <ul style="list-style-type: none"><input type="checkbox"/> Add all 3 pieces of raw Liver to the test tube.<input type="checkbox"/> Measure 6 ml of water into your 10 mL graduated cylinder. Add water to test tube with liver.<input type="checkbox"/> Label test tube with lab #<input type="checkbox"/> Bring test tube up to hot water bath<input type="checkbox"/> Heat until Liver looks completely cooked (will turn grayish brown) roughly 10 minutes should be enough.<input type="checkbox"/> Bring up test tube rack and use test tube clamp to take it out of hot water bath and set in rack. Let cool.<input type="checkbox"/> Dump water out of test tubes (when cool)<input type="checkbox"/> Put the three pieces of liver into a zip lock bag labeled “Method B: Boiled Liver” Also label it with your class period & Lab station number.<input type="checkbox"/> Add 6 mL fresh water to the bag<input type="checkbox"/> Wash Test tubes with water and test tube brush when completed<input type="checkbox"/> Seal the bag (Make sure to squeeze the air out of the bag first). |
| <u>Method C: Lemon Liver</u> | <u>Method D: Salted Liver</u> |
| <p>Using the graduated cylinder, funnel and lemon:</p> <ul style="list-style-type: none"><input type="checkbox"/> Place the funnel into the graduated cylinder<input type="checkbox"/> Squeeze as much lemon juice as you can out of the lemon into the funnel and collect it in the graduated cylinder until you have 6mL.<input type="checkbox"/> Pick out any seeds and throw them in the trash. When the lemon has no more juice in it, throw it in the trash. (If you have extra juice give to teacher)<input type="checkbox"/> If you could not get 6 mL of lemon juice, add water until you have 6 mL.<input type="checkbox"/> Put all three of your pieces of raw liver into a zip lock bag labeled “Method C: Lemon Liver” Also label it with your class period & Lab station number<input type="checkbox"/> Add your 6mL of Lemon Juice to this bag<input type="checkbox"/> Seal the bag (Make sure to squeeze the air out of the bag first). | <ul style="list-style-type: none"><input type="checkbox"/> Weigh 2 grams of salt, add to zip lock bag.<input type="checkbox"/> Measure 6mL of water, add to bag with salt.<input type="checkbox"/> Put three pieces of raw liver into a zip lock bag containing the salt water solution.<input type="checkbox"/> Label the bag “Method D: Salt Liver.” Also label it with your class period & Lab station number<input type="checkbox"/> Seal the bag (Make sure to squeeze the air out of the bag first). <u>Method E: Cold Liver</u> <ul style="list-style-type: none"><input type="checkbox"/> Get the bag “Method E: Cold Liver.” Also label it with your class period & Lab station number<input type="checkbox"/> Place 3 pieces of liver in it.<input type="checkbox"/> Do NOT add water<input type="checkbox"/> Seal the bag (Make sure to squeeze the air out of the bag first). |

*Make sure that all graduated cylinders are properly cleaned so that contamination does not occur!!

*Leave everything Cleaner than you found it!! Throw all trash Away

Step 4: Give all zip lock bags to Ms. Leyda.

(Make sure they are sealed completely with NO Air left in the bag.)

Liver Lab Day 2: Testing the Activity of the Enzyme

In Monday's lab you treated the enzyme peroxidase (found in liver) with 3 different methods: boiling, adding lemon juice and adding salt. You also left Method A and E Raw: A as a control and E to put on ice today to see if cold liver will affect the enzyme peroxidase. Today you will find out how each of these conditions affected the function of the enzyme. You will try to measure the activity of the enzyme in three ways: 1) Time the reaction in seconds, 2) measure the height of the reactions bubbles with a ruler in centimeters, and 3) take the temperature before the reaction leaving the thermometer in the liver for 2 minutes.

Procedure: Day 2

**** Method E will be done at the end: Teacher will keep in freezer UNTIL READY TO USE

1. Gather test tubes labeled A, B, C, D, and E.
2. Using the forceps take one piece of liver from zip lock bag "Method A: Raw" and take it's mass in grams and record on your data table for Trial 1: Method A.
3. After you have the mass recorded put the piece of liver into Test Tube A.
4. Repeat steps 2 and 3 for each of the other livers: B, C, D, and E, putting them in the correct Tube.

**Now you should have 1 piece of liver in each of the 5 tubes, and all masses should be recorded in the data tables

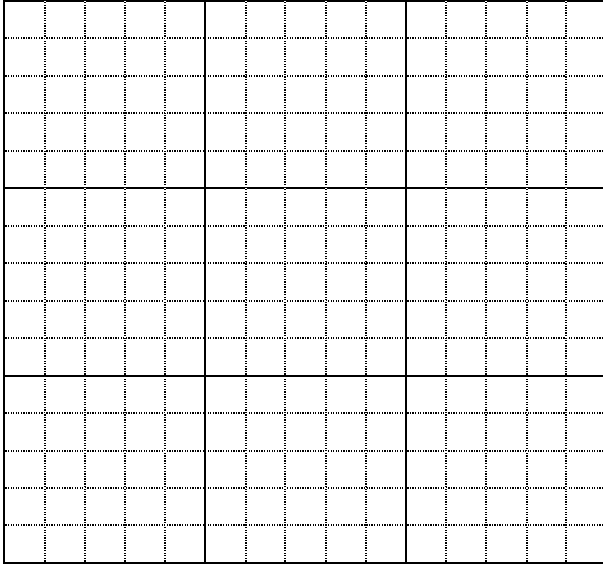
5. **Measure starting temperature of H_2O_2** in beaker (this temp should be the same throughout, but can check again halfway through to see if anything has changed).
6. Start with test tube A: Put thermometer into tube
7. Measure **2ml of H_2O_2** into your pipette labeled H_2O_2 .
8. Clear timer. Get ready to start timer!
9. Add H_2O_2 to test tube A: Liver. **Start** the timer as you do so.
10. **Stop timer when the reaction reaches its maximum temperature.** If the reaction stays at the same temperature for a long time, stop the timer when you get to 2 minutes and record "2 min plus"
11. Measure height of reaction with ruler: how high did the bubbles go up during the reaction? (**measure from the top of the liquid to the highest point of the bubbles**)
12. Repeat Steps 5-10 for Liver B, C, D, E. (note Tube E should be kept in the ice water while waiting)
13. **As you finish each trial with each tube** have someone in the group, or the "lab supply" person clean the test tubes with the test tube brush and prepare the sample for Trial 2. Trial 2 is repeating each of the steps (#2-11) all over again. This way you will get 3 trials of each of the 5 different treatments of liver completed.
14. Clean up! Leave everything neater than when you got to class.

Graphing:

Use your data to make graphs to compare the effectiveness of the different conditions on the peroxidase enzyme. Be sure to Title the graph and Label the X and Y axis.

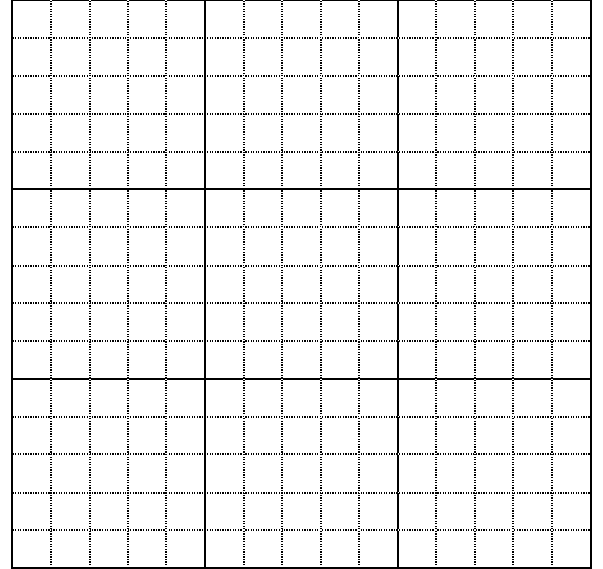
Graph 1: Graph the Average reaction rates/gram of liver

Title:



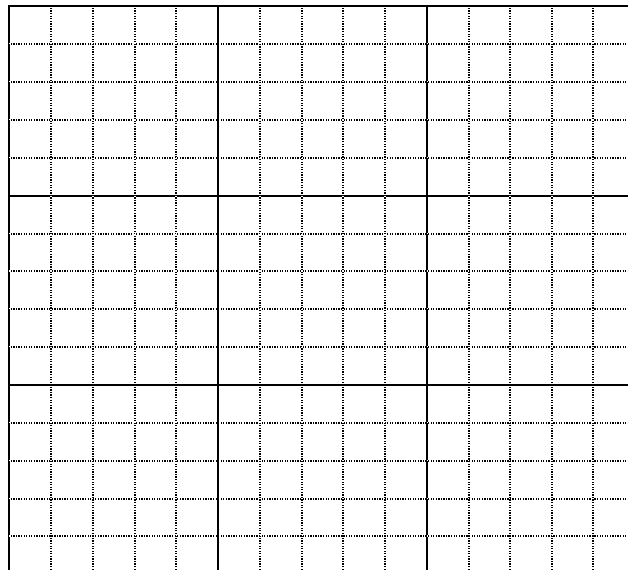
Graph 2: Graph the average time of reaction and average height of the reaction/gram on the same graph

Title:



Graph 3: Graph the average change in temperature of H_2O_2 /gram of liver and the height of the reaction/gram on the same graph.

Title:



Conclusion:

1. You took 4 different kinds of data in your experiments: temperature, mass of liver, time, and height of reaction. Which data do you think was the most accurate test of how well your enzyme was working to break down hydrogen peroxide? Why?

2. Using your data and comparing it to your control (raw liver): What effect did boiling have on the peroxidase in the liver? Make sure to use numbers from your data and use at least 5 of the following vocabulary words in your answer: active site, product, peroxidase, enzyme, co-enzyme, pH change, inhibitor molecule, temperature, or denature. HIGH LIGHT Vocabulary and DATA!!

3. Using your data and comparing it to your control (raw liver): What effect did the acid (lemon) have on the peroxidase? Make sure to use numbers from your data and use at least 5 of the following vocabulary words in your answer: active site, product, peroxidase, enzyme, co-enzyme, pH change, inhibitor molecule, temperature, or denature. HIGH LIGHT Vocabulary and DATA!!

4. Using your data and comparing it to your control (raw liver): What effect did the salt have on the peroxidase in the liver? Make sure to use numbers from your data and use at least 5 of the following vocabulary words in your answer: active site, product, peroxidase, enzyme, co-enzyme, pH change, inhibitor molecule, temperature, or denature. HIGH LIGHT Vocabulary and DATA!!

5. Using your data and comparing it to your control (raw liver): What effect did the ice water have on the peroxidase in the liver? Make sure to use numbers from your data and use at least 5 of the following vocabulary words in your answer: active site, product, peroxidase, enzyme, co-enzyme, pH change, inhibitor molecule, temperature, or denature. HIGH LIGHT Vocabulary and DATA!!

6. Controls and Sources of Error:

Explain all the things you controlled and all the things that went wrong or were not controllable, and what effect that might've had on results:
